Department of Defense Comments on Toxicological Review of Hexavalent Chromium		
Comments submitted by: Office of the Secretary of Defense Chemical and Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: May 5, 2010

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1.	2 *"Chemical and Physical Information"*	Global	Since Cr6+ and Cr3+ are the most common valence states of chromium naturally occurring in the earth's crust, we believe that it would be helpful if the text discussed the state-of-the-science related to analytical methods. It would be especially useful to discuss whether methods are available for the determination of Cr+6 in various environmental /biological media and in foods. This discussion detailing the potential analytical problems, detection limits and the uncertainties related to the analyses would be very useful and help explain why in many cases only total chromium was reported in some of the studies cited in the Toxicological Review. As an example, the recent Soares M.E. et al. 2010 paper entitled, "*Chromium speciation analysis in bread samples*," (*J. Agric. Food Chem.*, 2010, *58* (2), pp 1366–1370) discusses a new method to determine total and Cr+6 in bread samples. The authors' reported that the total chromium contents were 47.3 +/- 20.0 and 50.9 +/- 22.2 microgram/kg of dry weight for white and whole bread samples, respectively; those for Cr+6 were 5.65 +/- 5.44 and 6.82 +/- 4.88 microgram/kg of dry weight. These data show the difficulty in isolating potential impacts from Cr+6 at low levels of potential concern.	We recommend some discussion of the limitations of analytical speciation methodology for Cr+6 in environmental and biological media be included in this section as general chemical information. We believe this would provide the environmental community with information necessary to characterize the potential health risks from ingestion of Cr+6 and better explain why chemical speciation may be required instead of total chromium analyses of environmental media.	S

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2.	3.1. "*Absorption following ingestion*"; 6.1. "*Human Hazard Potential*."	9, lines 27-35; 10, lines 1-6; 204, line 22. NTP (2008).	Sutherland et al. (2000) reported elevated concentrations of chromium in various tissues with higher levels in bone and kidney after ingestion of Cr+6, demonstrating that chromium was taken up from systemic circulation (bioavailability of a portion of the chromium). The text states that the authors proposed two "*non-mutually exclusive*" possibilities to explain their results: (1) a portion of the ingested Cr+6 escaped reduction, entered systemic circulation, and was available for cellular absorption, or (2) the Cr+3 formed in the gut was absorbed and was not cleared by the kidneys but was taken up by the cells. As the chromium was reported as total chromium and not speciated, we question EPA's comment that "*In any event, this study suggests that even at relatively low concentrations, hexavalent chromium is likely absorbed and retained in the body*". It appears that EPA inferred this conclusion and not the study authors, but the text is not clear. Regardless, we believe that the word "*likely*" should be changed to "possibly" based on the authors' second proposal involving Cr+3 being absorbed and taken up by the circulatory system, and not Cr+6. Also, later sections of the EPA draft and the references therein indicate that Cr+6 is poorly absorbed via ingestion (only about 2-11%; Donaldson and Barreras, 1966; etc.).	Although we understand that the focus of this section is on the toxicokinetics of Cr+6 following ingestion, nevertheless, we believe it is important to describe alternate possibilities involving how Cr+3 can form complexes with organic ligands, which then allows Cr+3 to pass more easily across cell membranes and potentially enter systemic circulation and/or how the Cr+6 can be reduced inside the red blood cells to Cr+3. Thus, we recommend that the EPA text describing the opinions of these researchers (Sutherland et al., 2000) and others should <u>more clearly distinguish between the study researchers' conclusions and those from the EPA's</u> (page10, lines 5-6). As the species of chromium absorbed is dependent on various conditions, we believe that it is important to present alternate theories even if they differ from the EPA's final conclusions, especially as analytical speciation was usually not feasible. In keeping with a more realistic approach, we recommend that page 204, line 22 "*Human Hazard Potential*" should be reworded to indicate that not just "some" Cr+6 is reduced to Cr+3 in the GI tract, but that available data suggests that the majority of ingested Cr+6 is reduced to Cr+3 in the GI tract.	S
3.	4.2.1. *Subchronic*	43, lines 5-7; 49, lines 9-	Table 4-4 summarizes the hematological effects for **rats** exposed to Cr+6 in drinking water for 23 days	The inconsistencies in the text noted should be resolved. Moreover, we believe the NTP	S

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	Oral Exposure; NTP (2007, 2008).	14; 42-49; Table 4-4, 44-45, Hematological effects in male and female F344/N rats…up to 3 months; 45, Lines 7-8; Table 4-5, 50, Clinical chemistry effects in male and female F344/N rats…, 47; Table 4-7, 50. "*Incidence of none-plastic lesions observed in male and female F344/N rats.*"	to up to three months at various treatment levels. Page 43 states that although more severe, dose-related effects were observed in several hematological parameters, severity at 3 months was generally less than that observed at 23 days. The text does not mention that a number of the hematological parameters were within the limits of the experimental accuracy cited in the Table. We also noted variability in the direction of the changes in rat mean cell hemoglobin at 23 days compared to treatment time 3 months that was also not mentioned in the text. Lines 5-6 on page 43 only stated that MCH decreased. We believe it is noteworthy that <u>NTP (2007) considered the data to reflect a compensatory hematopoietic response.</u> Page 45 notes that "*A consistent relationship between severity and dose was not observed in the clinical chemistry results"* (T 4-5). This also appears to apply to Table 4-4. Page 46, lines 5-9 notes that NTP (2007) suggested that the clinical chemistry analyses' results indicate that exposure of rats to sodium dichromate dihydrate in drinking water induced hepatocellular membrane damage or cytotoxicity at doses greater than or equal to 1.7 mg Cr+6/kg-day. We believe that the hematological data from rats after 3 months compared to 23 days exposures help support NTP's (2007) conclusions concerning a compensatory response. Sex-related differences in whether organ weight increased (females) or decreased (males) were noted. NTP (2007) concluded that the changes in body weight	scientists who conducted the study are in a good position to review and interpret impartially the results, and therefore should at a minimum be presented alongside EPA's *post hoc* analysis. Specifically, the text on page 43 should be amended to support the actual data in Table 4-4, i.e., to better demonstrate the apparent compensatory mechanism after 3 months exposure in rats. The fact that the variations in hematological parameters were frequently within the range of experimental accuracy at 3 months should be noted in Section 4.6.3. This also applied to some of the responses for the data at 23 days (for example, mean cell hemoglobin (MCH) at 1.7 and 3.5 mg Cr+6/kg-day in male rats. This should be discussed in the text. The rationale for organ weight differences between the rat sexes at some treatment levels and exposure durations and the potential impact of weight loss, especially in the highest dosed animals, would be of interest as would other sex and/or species-related differences (NTP, 2007). We also recommend that the potential for a compensatory adaptive response related to increased hematopoiesis in response to Cr+6 exposure from ingestion be addressed in greater detail.	

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		.	may have impacted some organ weight changes. The text on pages 48 and 49 presents the significant histopathological changes noted from microscopic slide examination, to include "*In males [rats] a dose-dependent increase in the incidence of histocytic cellular infiltration of pancreatic lymph nodes was observed at 1.7 mg hexavalent chromium/kg-day, whereas increased pancreatic lymph node sinusoidal ectasia and lymphoid hyperplasia <u>were only increased in the highest dose-group</u>…*" (page 48, lines 18-19; page 49, lines 1-2). The text does not mention the positive control responses noted in the female rat for two effects and the lack of a dose-response for some of the endpoints noted in Table 4-7.		
4.	4.2.1. "*Subchronic Oral Exposure";* NTP (2007); 5.1.1.; NTP (2008).	54, lines 3-13; Table 4-9, 54. "*Incidence of non-neoplastic lesions observed in male and female B6C3F1…3 months*"; Table 4-11, 58.	The text states that based on histopathological changes **(**histiocytic cellular infiltration) in the duodenum, a LOAEL of 3.1 mg Cr+6/kg-day was identified for both sexes of B6C3F1 mice (3-month NTP (2007) study. Table 4-11 in the EPA draft (NTP, 2007) provides the incidence of nonneoplastic lesions observed in another mouse study comparing effects in three different strains of mice, one of which was the same B6C3F1 strain. It is interesting to compare the results of the study using B6C3F1 mice given in Table 4-9 with the same endpoint (duodenum (histiocytic cellular infiltration) and same strain (B6C3F1) of mouse from the comparative study depicted in Table 4-11.	Although we acknowledge that in both the 3-month and two-year NTP toxicity studies (NTP, 2007a; 2008) of sodium dichromate dihydrate, histiocytic cellular infiltration was consistently observed in several tissues including the liver, duodenum, and mesenteric and pancreatic lymph nodes of rats and mice, we believe that the increased response in the same mouse strain (B6C3F1) depicted in Table 4-11 compared to Table 4-9 (NTP, 2007) subchronic studies) deserves attention and should be acknowledged and discussed in the appropriate section(s) of the EPA draft. EPA may wish to consider including this apparent	S

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		"*Incidence of non-neoplastic lesions observed in male B6C3F1, Balb/C, AND AM3-c57bl/6 mice …3 months*"; 186-186.	Of the three strains of mice, the 4/10 number of positive lesions observed at 3.1 mg Cr+6/kg-day (LOAEL) (Table 4-9) for mouse strain B6C3F1 had fewer lesions than at the lower treatment dose of 2.8 mg Cr+6 /kg-day LOAEL given in Table 4-11, which reported 8/10 nonneoplastic lesions. Both studies were for 3 month durations. A similar comparison for the next higher treatment group of B6C3F1 mice yields a similarly great difference in the number of lesions reported between the two studies. The reproducibility of the results for the lower level treatment groups does not lend to their suitability for quantitative analyses. This variability in the number of positive findings should be addressed in the appropriate sections of the text where the results are discussed (and the uncertainty analyses) and may impact the ability to draw meaningful conclusions from the lower concentration response data.	inconsistency in the uncertainty section and indicate whether these variations may be of significance, particularly as increased incidence of histopathological changes in the duodenum in both male and female mice were considered as possible critical effects in derivation of the oral reference dose based on chronic exposure (NTP, 2008).	
5.	4.4.1. "*Genotoxicity Studies*"; 7.0. "*References*."	115, lines 11-14. NTP, 2008; Wise et al., 2008; 2010; NJ, "*Derivation of Ingestion-Based Soil Remediation Criterion for*	Page 115 discusses the mutagenicity of Cr+6 and states that it is mediated through the generation of highly reactive chromium intermediates and reactive oxygen species, citing Wise et al., 2008. It was our understanding that the postulated mechanism(s) underlying chromium-induced genotoxicity and carcinogenicity is still the subject of much research and some scientific debate (NTP, 2008). The Wise et al. paper cited states, "*In the particulate form, Cr(VI) dissolves slowly in vivo, leading to an extended exposure of lung cells…Hexavalent chromium is taken into the cell and rapidly reduced*	We believe that the text on page 115 should indicate that the authors believed the Cr+6 was inhaled as particulate. EPA should also consider how this might affect its conclusion regarding mode of action of carcinogenicity, as well as conclusions regarding absorption. We acknowledge that there must be a cut-off date for considering publications for inclusion in the Toxicological Review, but EPA may wish note that the following additional, more recent publications: Wise et al., 2010, "*Chronic exposure to zinc chromate induces*	S

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		Cr^{+6} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate" **(**April, 2009).	*to Cr(V), Cr(IV), Cr(III), and reactive oxygen species. Cells treated with Cr(VI) are subject to several types of DNA damage resulting from this reduction…These types of damage, if left unrepaired or are misrepaired, can lead to growth arrest, cytotoxicity, and apoptosis, as well as mutations leading to neoplastic trans-formation and ultimately tumorigenesis.*" (emphasis added) Thus, the Wise et al. (2008) study appears to suggest a sequential progression of events that may lead to tumor formation after long-term inhalation exposure of particulate. This is in contrast to the rapid absorption of a small portion of the ingested Cr+6 and its known reactivity at the site of contact (GI tract). A more recent paper by Wise et al.(2010), entitled, "*Comparative genotoxicity and cytotoxicity of four hexavalent chromium compounds in human bronchial cells***,"** also discusses the importance of solubility in Cr+6 to its carcinogenic potential and states that particulate Cr+6 compounds slowly dissolve in the lung and are thus the most carcinogenic. Although no studies were located regarding genotoxic effects in humans after oral exposure to Cr+6, recent examinations of chromosomal damage after *in vivo* exposures of animals of Cr+6 in drinking water have given mixed results (DeFlora et al., 2006; NTP 2007a). Although the referenced documents cite numerous studies providing experimental evidence in support of the role of reactive oxygen species in the genotoxicity of chromium, other studies raise	*centrosome amplification and spindle assembly checkpoint bypass in human lung fibroblasts;"* Soares, M.E. et al, 2010, *Chromium speciation analysis in bread samples; and* Wise et al.(2010), *"Comparative genotoxicity and cytotoxicity of four hexavalent chromium compounds in human bronchial cells.***"**	

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			questions about the relative contribution of this mechanism (O'Brien *et al.*, 2003; Zhitkovich, 2005; Quievryn *et al.*, 2006). In particular, if chromium mutagenicity is mediated through reactive oxygen, it would not be considered a mutagenic mode of action, as the chemical (or its metabolite) would not be directly mutagenic. NTP (2008) concluded that "*There was evidence of systemic exposure to Cr VI following oral administration in the drinking water based on the tissue distribution data, toxicity to the hematopoietic system, and the presence of microscopic changes in multiple tissues*." Advances in analytical detection methods involving speciation of total chromium may help resolve this scientific debate.		
6.	Table 4-23. "*In vivo genotoxicity studies of hexavalent chromium…*";	142	The table incorrectly labels the second study reported in *NTP Technical Report on the Toxicity Studies of Sodium Dichromate Dihydrate* (NTP, 2007) as "±" for the B6C3F1 mice. Although the text of that report says that the results were "*[a]n equivocal increase*," that same sentence concludes "*based on a small increase in micronucleated normochromatic erythrocytes that did not reach statistical significance*." Following standard practice, an increase that is not statistically significant should be summarized as negative, "–".	The notation in the table should be corrected, as well as any references to this as an equivocal rather than a negative result.	S/M
7.	4.6.3. "*Mode of Action*	Global; 134-143;	The document correctly notes that *in vivo* mutagenicity by oral exposure in mice is highly dependent on the strain used. After correcting the	The mode of action analysis for the tumors used for deriving the cancer potency should be changed, as the data for this strain of mouse	S/M

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	Information"	Table 4-23, 142. "*In vivo genotoxicity studies of hexavalent chromium…*" ; Table 5-5, 196. "*Incidence of adenoma and carcinomas combined in the small intestine of male B6C3Fi mice…*"	notation for the results of two studies in the male mice in the strain used for cancer studies (see comment #6 on Table 4-23, below), <u>this strain does not produce mutations by the oral route.</u> Therefore, the biological plausibility is lacking for a mutagenic mode of action for the tumors produced, as oral exposure to Cr+6 in these mice does not produce mutations. The ability of a chemical to cause mutations in some biological systems is necessary, but not sufficient to establish a mutagenic mode of action for carcinogenesis. In this case, we are fortunate to have *in vivo* mutagenicity and cancer bioassays performed in the same strain of mouse by the same Federal testing facility, thus obviating considerations of both quality of testing, genetic drift of inbred strains, and quality of analysis of the results. The conclusion is clear: <u>The tumors produced in the male B6C3F1 mice can not have a mutagenic mode of action because this strain of mice did not produce mutations under the same conditions tested at the same time in other strains of mice.</u>	can not support that finding. An alternative, plausible mode of action for these tumors is proposed in comment #3, although time will not allow us to perform the analysis to see if the data will support that mode of action. If the data support that mode of action, it would be reasonable to use a nonlinear extrapolation from the point of departure, as the mode of action requires high concentrations of the chemical at a point proximal to that of exposure. A nonlinear, low-dose extrapolation is further supported by the lack of statistically significant tumors at the first two doses of the experiment (Table 5-5).	
8.	4.6.3.1. "Hypothesized *Mode of Action*."	168, line 32; 169, lines 18-20.	If "*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*," it is not completely evident why overall Cr+3 less carcinogenic. In particular, EPA's IRIS analysis of Cr+3 states, "*The data from oral and inhalation exposures of animals to*	The document should explain why, if its mutagenic mode of action depends on the reduction of Cr+6 to Cr+3, the latter is less carcinogenic by both routes of exposure, and also less mutagenic. If EPA's current conclusions regarding Cr+6 depend on an updated analysis of Cr+3, the two would	S/M

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			trivalent chromium do not support determination of the carcinogenicity of trivalent chromium. IARC (1990) concluded that animal data are inadequate for the evaluation of the carcinogenicity of Cr (III) compounds… In general, trivalent chromium was not mutagenic in bacterial assays when tested with or without a mammalian activation system (Venitt and Levy, 1974; Petrilli and Deflora, 1977, 1978a,b). In one study, trivalent chromium was mutagenic in Baccillus subtilis, but this activity was low compared with compounds of hexavalent chromium (Nakamuro et al., 1978)." (emphasis added). As currently written, differences between Cr+3 reduced from Cr+6 activity versus administered Cr+3 are not clear in the document. These differences should be clearly described in order for risk managers and the public to have a clear understanding of the proposed mechanism, especially since chromium is an essential nutrient.	optimally be reviewed together. Conversely**,** if the differences in carcinogenicity are due to differences in cellular absorption of Cr+6 and Cr+3 it should be more clearly described. If there are differences in mutagenic activity of Cr+3 when it enters the cell with an organic ligand it should be described.	
9.	4.6.3.2 "*Experimental Support for the hypothesized Mode of Action";*Table 4-23; Section 4.2.1, *"Subchronic*	55, line 10; 142 and 171, line 21	The text in section 4.2.1 states that though five am3-C57BL/6 mice were exposed to sodium dichromate dehydrate for a mutagenicity study, but the studies were not carried out due to technical problems. This seems to be in conflict with the results reported in Table 4-23 and the results described in Section 4.6.3.2.	The conflicting text needs to be corrected or the text in Section 4.2.1 needs to be made more clear.	S,E

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	oral Exposure"				
10.	4.6.3. "*Mode of Action Information*"; 5.1.1, "*Choice of Principal Study and Critical Effect*."	168-181; 186	EPA should reconsider its conclusion that Cr+6's potential carcinogenicity is related to a mutagenic mode of action, specifically via an oral exposure pathway. The results of NTP and others suggest that a site of contact (or chromium accumulation) produces cell damage, progression to cytotoxicity, and regenerative proliferation as a more plausible mode of action. Page 174, lines 19-24, state that "*Only one study examined tumor target tissue for evidence of mutagenicity (De Flora et al., 2008). De Flora et al. (2008) found negative results for DNA-protein crosslinks and DNA adducts in the duodenum in mice following drinking water exposures. Other available drinking water exposure studies of hexavalent chromium that measured mutagenicity in mice failed to show evidence of micronucleus induction in the blood or bone marrow (De Flora et al., 2008, 2006; NTP, 2007; Mirsalis et al., 1996).*" The following excerpt is from NTP (2007): "*Regarding other evidence for systemic or site of contact toxicity of exposure to sodium dichromate dihydrate, a significantly increased incidence of ulcer and epithelial hyperplasia and metaplasia of the glandular stomach occurred in rats in the 1,000 mg/L group. Coincident with the focal ulcers was evidence of inflammation within the stomach wall. The increases in blood neutrophil and monocyte counts were consistent with an inflammatory*	EPA should reconsider its evaluation of the strength of evidence for a mutagenic mode of action for carcinogenicity specifically related to oral exposure via the ingestion exposure pathway, "*within the reductive capacity of the GI tract*" based on the likelihood of alternate modes of action, as presented in the cited references and discussed in the preceding comments pertaining to data and rationale suggestive of an alternate mode of action. We note that the New Jersey Department of Environmental Protection's risk assessment (on which EPA states they based their Cr+6 dose/response; Forward, page *x*) recently concluded that the NTP data indicated other alternate modes of action related to potential oral carcinogenicity of Cr+6 (April, 2009). As an aside, it would be helpful if EPA clearly state the results of the "key" studies in an "Executive Summary." This would help identify which endpoints, species, studies EPA considered critical and thus, were used to help EPA derive their Cr+6 proposed oral cancer potency value and other toxicity values for IRIS.	S, E

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			response. These lesions occurred at the junction between the forestomach and the glandular stomach, where the reduction of Cr VI to Cr V and to Cr IV and subsequent radical formation would be enhanced by the increased acidity. Histiocytic infiltration was consistently noted as a minor lesion in the duodenum of the small intestine of rats and mice receiving sodium dichromate dihydrate. Hyperplasia, characterized by villi that were taller than normal and with tightly packed basophilic epithelial cells, was an additional finding in the small intestine in all strains of mice." These findings are characteristic of a non-mutagenic mode of action.		
11.	4.6.3.2. "*Experimental Support for the Hypothesized Mode of Action*."	170, line 30	The *in vivo* section emphasizes the positive results in fruit flies, while only mentioning afterwards the inconsistent results in mammals.	We suggest discussing the mammalian data first, as these data are more relevant for the tumors of interest, in particular, the species-dependent results.	E
12.	4.6.3.3	Global	As organs affected by the cancers are directly associated with the route of exposure, it would seem that the cancers are likely to be caused by a high (bolus) dose proximal to site of exposure. This is also consistent with the findings that (1) the mutagenicity and carcinogenicity are associated with high levels of exposure and not at lower levels of exposure.	This alternative mode of action, i.e., that hexavalent chromium is carcinogenic only at high levels of exposure, should be discussed in section 4.6.3.3. "*Other Possible Modes of Action.*" Ideally in the absence of a known mode of action, if sufficient biological information exists to infer that the chemical may be nonlinear at low doses, both procedures (linear and nonlinear extrapolation) would be	S

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				presented as part of risk characterization. If EPA does not provide this information in this section, it can not easily be brought forward into the risk characterization. We therefore recommend that EPA also provide a nonlinear extrapolation from the point of departure in addition to the linear extrapolation.	
13.	5.1.1 "*Chronic Studies*"; 4.6.3.3. "*Other Possible Modes of action*"; NTP (2008)	185, Lines 1-27; Table 5-1, 187. "*Incidence Data for Lesions From All Treatment Groups of Female F344/N Rate and Male and Female B6C3F1 Mice….*"; 179.	Page 185 states that NTP (2008) study identified "*No Observable Adverse Effect Level*" (NOAEL) and "*Lowest Observable Effect Level*" (LOAEL) values for noncancer effects in male rats of 0.21 and 0.77 mg Cr+6/kg/day, respectively, based on increased incidences of nonneoplastic histopathological changes to the liver, duodenum (histiocytic cellular infiltrate), and mesenteric lymph nodes. In female rats, a LOAEL for noncancer effects of 0.24 mg Cr+6 /kg-day was identified based on the increased incidence (21/50) of chronic inflammation of the liver (observed in all treatment groups). Lines 7-8 state that a NOAEL was not identified because effects observed were at the lowest dose tested. The text does not mention that 12/50 of the control female rats also showed chronic liver inflammation, as shown in Table 5-1. An effect is a NOAEL if it is not observed to have a statistically higher incidence in dosed animals than in control animals. EPA should determine if this is the case. This study identified a LOAEL for noncancer effects of 0.38 mg hexavalent	We suggest that this section provide the control positive response data from the NTP (2008) study, particularly for the male mice, and compare them to the number of mice with positive histopathological responses attributed to the toxicity of Cr+6, as the positive mice data (and not the rat data) were considered "critical" to EPA's derivation of their proposed Cr+6 ingestion-related toxicity values. The potential effects that may not be directly related to the toxicity of chromium *per se* should also be included, such as the potential impact of the palatability of the drinking water and weight changes, as applicable, should be discussed, as well as other sex-related differences noted that may impact the results should be discussed in greater detail (NTP, 2008). Also, the potential for formation of hyperplasia. resulting from cytotoxicity without progression to GI tract tumors in rats	S, M

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			chromium/kg-day in both male and female B6C3F1 mice; a NOAEL value was not identified because effects seen were at the lowest dose administered. Table 5-1 also showed that 14/47 of the control male mice were positive for histiocytic cellular infiltration. In males, the LOAEL was based on increased incidences (11/50) of histopathological changes to the duodenum (diffuse epithelial hyperplasia) and mesenteric lymph nodes (histiocytic cellular infiltration); in females, the LOAEL was based on increased incidences of histopathological changes to the duodenum. Again, EPA should determine if the response in the dosed animals is a NOAEL rather than a LOAEL, given the high level of responses in the control animals. The NTP (2008) 2-year study involved the use of multiple dose groups, and included a comprehensive evaluation of multiple endpoints. Also, this bioassay used lower doses than the subchronic (90-day) studies also conducted by NTP (2007), and thus provides dose-response information at lower exposure levels than the 90-day studies. When the adenomas and carcinomas were combined for all sites of the small intestine, including the duodenum, jejunum, and ileum, there was a clear exposure response relationship, and the incidences were statistically significant in the two highest exposure groups of male and female mice.	compared to mice data (NTP, 2008) should be discussed in greater detail in section 4.6.3.3 of the document on alternate modes of action (page 179). We recommend that this section should be expanded to include the potential link between noncancer and cancer effects in mice and the potential role of cytotoxicity for both species in the GI tract, as it relates to an alternate mode of action, should be discussed in greater detail.	
14.	5.1.1	189	The choice of diffuse epithelial hyperplasia of the	The possibility that duodenal lesions in mice	S,M

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			duodenum in female mice as the critical effect is questionable because of decreased drinking water intake due to decreased palatability of chromate-tainted water (see Page 75 para 2). Decreased water consumption and resulting abrasion of the luminal lining on ingestion of pelleted dry rodent chow is a possible cause of the observed duodenal lesions following chromate administration in drinking water. (see DeSesso, J.M., A.L. Lavin, S.M. Hsia, and R. D. Mavis. 2000, "Assessment of the Carcinogenicity Associated with Oral Exposures to Hydrogen Peroxide," Food and Chemical Toxicology, 38, pp. 1021-1041.) If the duodenal lesions are a result of abrasion of the lumen by dry rodent chow, this effect is not appropriate for derivation of a human RfD.	arose from abrasion of the luminal lining by dry rodent chow due to decreased water consumption should be discussed; the impact of this possibility on the appropriateness of this effect as the critical effect should be addressed. A different critical effect should be chosen if this possibility cannot be eliminated.	
15.	5.3.3. "*Dose Adjustments and Extrapolation Method(s)*."	197, line 7.	The statement that "*The dose response is assumed to be linear in the low-dose range when evidence supports a mutagenic mode of action because of DNA reactivity*" is not accurate. Our search of the cancer guidelines shows it states: [emphasis added] "Agents that are <u>generally considered</u> to be linear in this region include: • agents that are DNA-reactive and have direct mutagenic activity." These cancer guidelines also state "*Special attention is important when the data support a nonlinear*	The sentence should be revised so that "*assumed*" is replaced by "generally considered".	S

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			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 at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches." Thus, our read of the cancer guidelines is that it allows for the occurrence of tumors by a chemical that can cause mutations but does not act by a mutagenic mode of action and/or that are nonlinear at low doses even with mutagenicity.		
16.	General, Sections 5 and 6	Global	Page 66 of the NTP (2008) report states that the rat oral mucosa carcinomas were highly aggressive neoplasms that invaded other tissues, such as the soft tissue around the nose. The human relevance of the rat squamous epithelial cell carcinomas of the oral mucosa and tongue, etc., from ingestion of drinking water containing Cr+6 should be discussed even though these data were not used to derive the oral cancer slope factor. NTP also stated that these carcinomas are relatively rare in rats and were not seen in the mouse studies. The National Cancer Institute (NCI) has stated that oral cancer accounts for about 3% of cancers in men and 2% of cancers in women. Thus, it does not appear to be a common human cancer.	We recommend providing epidemiological data concerning the statistical incidence of the oral cancers discussed to help risk assessors relay the current estimates for these types of cancer in humans to risk managers and the concerned public who may misinterpret the need for chromium as an essential nutrient based on the rodent cancer data presented. This would be consistent with the "reality check" proposed by EPA's SAB when reviewing EPA's toxicity evaluation of arsenic, where the SAB member indicated that EPA's projection of human lung cancers due to arsenic would exceed those caused by smoking.	S