National Institute of Environmental Health Sciences
Comments on the Interagency Science Consultation Draft
EPA IRIS Toxicological Review of Hexavalent Chromium [Cr(VI)] [CASRN 18540-29-9]
Dated February 2022

Date: 03/28/2022

Note: The technical review and comments are provided by the National Institute of Environmental Health Sciences (NIEHS), cleared internally, and are not intended to represent any agency position or opinion.

1 Introduction
- 1.1. Overview
- 1.2. Summary of Assessment Methods
  - 1.2.1. Literature Search and Screening

NIEHS Comments: EPA's IRIS program has clear procedures and generally transparent methods, including recent methodological advances and release of the IRIS handbook. It is understandable that Toxicological Reviews such as Cr (VI) take time and that both methods and documentation standards may have evolved in parallel over the life of a review. To help reviewers it would be useful to include the protocol with review material and to clarify if the protocol as posted in 2018 is current or in process of being updated. If it is being updated, completing that update in advance of further review activities is recommended, if feasible. Given the available materials, additional documentation of the literature search strategy would help peer reviewers in assessing the adequacy of the search. The screening process is described well in the protocol (Protocol - **page 18**).

- A specific listing of the search strategy with search terms and structure for each database would help with the transparency of reporting. It is not clear if that information is provided in the methods, supplemental material, or protocol. If so, then the addition of a clear link to the specific section with the search strategy would support review and prevent concerns as to the adequacy of the search.
- Addition of a brief description of the screening process in the main document would be helpful and appears to be missing from the Section 1.2.1 of the main document.
- Evaluations take time as does the review process, and so the last date of literature search of October 2019 is understandable. EPA should consider if an updated search could be conducted because over 2 years represents a significant time lag for supporting a current review.
- Providing additional clarity on the dates of the literature search would be helpful to support transparency and prevent concerns as to the adequacy of the search.

- Literature Search and Study Evaluation Results
  - Literature Search and Screening Results
  - Study Evaluation Results

NIEHS Comments: No comments for section 2.2 as the majority of the information is presented in Section 3 with specific details by health outcome categories.

3. Hazard Identification

3.1 Overview of Pharmacokinetic

NIEHS Comments: The studies used, and the conclusions made with regards to ADME following oral or inhalation exposure to Cr (VI), are appropriate. Overall, the information is clearly presented, the decisions are well described, and scientifically justified. As noted, characterizing the speciation of Cr following exposure is challenging; therefore, the assumptions applied for hazard characterization and mode of action analysis utilizing total Cr value are justifiable. The blood and tissue concentrations of total Cr measured in studies following oral exposure to Cr (VI) are likely resulting from a combination of rapid active cellular uptake of administered Cr (VI) that was absorbed, slow cellular uptake Cr (III) via diffusion that was absorbed as Cr (III) from extracellular (e.g., gastric juices) reduction of administered Cr (VI), and slow cellular uptake of Cr (III) that was formed from absorbed Cr (VI) via reduction by the liver or other components in the body (e.g., plasma and red blood cells).

3.2. Synthesis and Integration of Health Hazard Evidence by Organ/System

3.2.1. Respiratory Tract Effects Other Than Cancer

3.2.1.1. Human Evidence

NIEHS Comments: Page 3-20, line 21: This text appears to indicate that in 3 of 4 studies (Li, et al; Kuo, et al; Lindberg and Hedenstierna), there was potential for residual confounding in other studies. The text stated that concern was raised in the Sobaszek study due to a lack of air or biomarker measurements… (lines 19-20). However, on lines 24-26, the text states that in all the studies, co-exposure may also contribute to observed health effects. It is unclear if 3 of 4 of the studies or all of the studies were of concern because of potential co-exposure.
Page 3-24: It appears that the text starting on line 5 is a repeat of text starting on line 1 of page 3-23.

3.2.2. Gastrointestinal Tract Effects Other than Cancer

3.2.2.4 Integration of Evidence

NIEHS Comments: Page 3-58, Lines 10-12: The text states "Cr(III), the reduced form of Cr(VI), is not a substrate for active transport through the cell membrane and would therefore enter cells through passive diffusion or phagocytosis (Witt et al., 2013). Witt et al. is a review article; therefore, citing the original publication is recommended. (e.g., a reference by Proctor et al. 2002 cited in Witt et al. may be appropriate or other suitable references.)

3.2.3. Cancer

NIEHS Comments: The document is based on a comprehensive review of the available scientific literature. The findings in this section are clearly and accurately summarized and presented, and the scientific interpretations are objective and reasonable based on the references that the authors reviewed and summarized. The protocols/methods are clearly described/summarized with the appropriate level of detail. There are no significant scientific criticisms.

However, there is perhaps a minor, but important, detail that should be corrected in the interest of being anatomically correct. Several statements, as written, suggest that the small intestine and the duodenum (which is the proximal part of the small intestine) are proximal to the stomach (see highlighted sections below). This text is somewhat misleading. Anatomically, the small intestine as a whole begins immediately distal to the stomach and the duodenum is

the first or proximal segment of the small intestine that connects to the stomach. These sentences should be edited to indicate that the tumors occur in the areas of the small intestine or duodenum "that are immediately distal to the stomach".

- **Page 3-46**: In animal studies, the areas of the small intestine that are more proximal to the stomach (the duodenum and jejunum) appear to be more susceptible to injury than the ileum, the distal portion.
- **Page 3-71**: In the same study, male and female B6C3F1 mice exhibited increased incidences of adenomas and carcinomas in the small intestine, with most tumors occurring in the duodenal section most proximal to the stomach.
- **Page 3-123**: The highest incidences of tumors and potentially preneoplastic lesions were observed in the duodenum, the region most proximal to the stomach.
- **Page 3-124**: All analysis was performed in the middle section of the duodenum, which may be a significant source of bias because ingested Cr (VI) tissue concentrations are expected to be highest in the section of the duodenum closest (proximal) to the stomach….

3.2.3.2 Gastrointestinal Tract Cancer

NIEHS Comments: Human Evidence via Oral Route of Exposure: The text is clearly written, and the assessment of the studies and evidence is adequate**.**

Overall comments

- Meta-analysis is a reasonable approach for evaluating a possible link between occupational exposure to hexavalent chromium and the four types of cancer.
- The EPA meta-analyses are not very informative for evaluating the potential association between chromium exposure and these cancers because the analyses include many studies of workers who were exposed to multiple carcinogens and do not have risk estimates that are specific for chromium exposure or are for chromium exposed subgroups. Although the forest plots depict risk estimates stratified by occupational groups, some groups combine studies with varying degrees of certainty of chromium exposure. For example, the chromate production group includes studies of stainless-steel workers (e.g., Moulin 1993). The chromium pigment production group includes some general painting studies. Moreover, the meta-analysis does not provide separate risk estimates for the different occupational groups.
  The recommendations for study inclusion/exclusion (generally follow the IARC monograph on hexavalent chromium, 100F guidance) are presented in the following table:

Include/exclude	Industry	Comments
Include (Currently included)	Chromium production workers	These studies are included except Satoh 1981.
Include (Currently included but too broad)	Chromium pigment production workers	Do not include general studies of painting unless significant chromium exposure is clearly documented (e.g., Dalager 1980).

Include/exclude	Industry	Comments
Include (Currently included)	Chromium electroplating	
Include (Currently included)	Chromium specific estimates	e.g., Boice, Lipworth
Include (Currently included but too broad)	Ferrochromium	Exclude the studies by Huvienen and Pukkula because they do not provide risk estimates for chromium exposed subgroups for the four cancer sites
Exclude (Currently included) Possibly include	Welders (mixed) E.g., Hansen Stainless steel welders with documented significant Cr exposure, e.g., Sjögren et al. (1987)	"Welding fumes" is a known human carcinogen. Exposure to chromium and nickel does not explain all the excess lung cancer risk of welding, e.g., risk estimates in mild steel welders are similar to estimates in stainless steel welders. Most analyses looking at Cr exposure in welders were limited to lung cancer.
Exclude (Currently included)	Tanners	Exposure is to a mixture of carcinogens, such as dyes and formaldehyde. Chromium exposure is not always documented.
Exclude (Currently included) Possibly include	Cement production workers Mason workers: Rafnsson 1997 (Cr measured in urine) Unclear: Jakobssson 1993	Exposure is to a mixture of carcinogens, e.g., silica and PAHs. Ferrous sulfate was added (beginning at a certain date) to reduce chromium (VI) to Chromium (III). Chromium exposure is not always documented.
Exclude (Currently included)	General printing/painting, nickel plating/stainless steel. Examples: Morgan	Exposure is to a mixture of carcinogens. Chromium exposure is not usually documented.
Exclude (Currently included)	Chromium exposure is not specifically documented in the study population, e.g.,	The predominant exposure in the Delzell study is probably metalworking fluids.

Include/exclude	Industry	Comments
	Delzell, Garabrent and Wegman, Ramanakumar, Kaerley, Olsen	

Other recommendations:

- Rate revised included studies based on EPA's comprehensive systematic review procedures (e.g., selection bias, information bias, confounding, sensitivity, etc.).
- Revise the meta-analysis using more specific inclusion-exclusion criteria, conduct subgroup analyses for the specific occupational groups, and conduct sensitivity analysis based on study evaluation rating.

Reporting recommendations:

- Provide greater details on the studies, their assessment, and the meta-analyses.
- Move the information on the meta-analysis from the supplement to the main document. Table 3.13 (in the main document) on meta-analyses in the peer-reviewed literature is not very informative (as EPA conducted their own meta-analyses) and could be removed or moved to the supplementary document.
- Provide a table of all the studies included in the meta-analysis for each cancer site with the following information: characteristics of the study population, industry, exposure assessment, outcome, study evaluation, risk estimates (used in the meta-analysis as well as any other relevant estimates not used in the meta-analyses.

NIEHS Comments: Animal Evidence via the Oral Route of Exposure

- Page 3-72: In male mice, there was a significant trend for jejunal adenomas, and carcinomas occurred in low incidences in some exposed groups, but not the control group. This should be mentioned in the text. At present, only female jejunal neoplasms are discussed.
- Page 3-73: Squamous cell papilloma of the tongue should be added (incidences were 1, 1, 0, 0, 0) for female rats.
- Table 3-15: Need to include squamous cell papilloma (tongue) for female F344 rats (1, 1, 0, 0, 0); footnote c should be corrected accordingly. The footnote says "(i.e., there were no squamous cell papillomas in the oral cavity of female rats.) (Reference: NTP TR 546 page 43).
- Page 3-74, lines 8-9: Document states that "The historical controls for squamous cell carcinoma of the tongue were 0/1298 for male rats and 1/1350 for female rats (see Appendix D.5). These data are not included in the NTP Technical Report (TR, page 44) or in the referenced Appendix. A reference is needed.
- Page 3-74, lines 9-11: Text states that the historical rates of squamous cell carcinomas and papillomas in the whole oral cavity in rats are less than 1% in both males and females. However, the NTP TR (page 44) cites rates of 0.3% (males) or 1.2% (females). It is unclear if these rates were determined in a manner separate from that reported by the NTP.
- According to the table in the NTP TR (page 44), the rates were 0.3% for males (footnote i) and 1.2% for females (footnote k).
- Page 3-74, lines 13 and 14: Text states that "Tumors of the small intestine of mice are also rare (historical rates of 2.3% and 0.67% in 13 males and females, respectively)." However, the NTP TR cites rates of 3.7% and 1.1%, respectively, for males and females

(Page 60). It is unclear if these rates were determined in a manner separate from that reported by the NTP.

- According to the table in the NTP TR (page 60), the rates were 3.7% (footnote o) and 1.1% (footnote v).
- The historical control rates are not provided in Appendix D and appropriate references are needed.

3.2.3.3. Mechanistic Evidence (all routes)

NIEHS Comments: The information in section 3.2.3.3 is very concise without omitting essential information needed to understand the content of the review, and the information is presented in a clear and organized manner. Analysis of the strengths and weaknesses of each study appears to have been conducted objectively, based on the principles of systematic review and with consultation of OECD test guidelines where available. Conclusions are reasonable and supported by the literature. There are no scientific criticisms for section 3.2.3.3.

The information in section 3.2.3.3. is clearly presented and there is an adequate level of detail for the methods and protocols. However, the use of the key characteristics is problematic when it is applied to in vitro data that is not dose contextualized to the in vivo studies. The reason for this is that nearly any chemical can elicit a cell stress response and nearly every other key characteristic once a high enough dose is achieved in vitro, hence it would likely be appropriate to caveat the evidence of effects for key characteristics that are derived purely from in vitro data that have not been kinetically scaled to in vivo exposures. Also, in relation to the in vitro data supporting the key characteristics findings, it is notable that supportive evidence can be derived from transformed cell lines and in certain cases there is not a tissue correspondence between the cell line and the tissues affected in vivo. It is understandable why these data are included; however, it would be helpful to the reader to make it clear that the in vitro system represents a general biological sensor of the effect and is not necessarily representative of target tissues, hence there is lower certainty of the in vivo relevance. Short of providing in vivo contextualization both from cell type and tissue concentration, a footnote should be added that in vitro associations with the key characteristics are potentially spurious and, therefore, have lower certainty.

Minor comments:

- **Page 3-106, lines 3–6:** Consider noting that for the evaluation of micronuclei in mature erythrocytes, a minimum of 4 weeks is recommended for micronuclei in mature erythrocytes to reach a steady state when a repeat-dose study design is used.
- **Page 3-105, lines 18–20:** Hypothetically, there could be instances in which, under conditions of an MTD, a test agent could reach bone marrow (a highly perfused tissue) but is not toxic to the bone marrow.
- **Page 3-113, lines 8–26:** It may also be worth noting that cells with DNA damage may have died, but O'Brian et al. (2013) did not observe any increases in cell death (apoptosis or necrosis) in crypt cells.

3.2.3.4. Mode-of-action Integration of Evidence for Carcinogenesis (Wang)

NIEHS Comments: Section 4.2.3.4 provides detailed background information and explains the differences of similar changes, which are helpful for readers to understand the

implication/importance of the specific results (for example, **Page 3-138**, lines 5-8). Such transparency and explanations should be added throughout the section (e.g., including rationales of seemly contradictive observations – such as on **Page 3-135**, lines 21- 27: increased apoptosis and evasion of apoptosis).

For accuracy of subheaders related to key characteristics, indicate when the effect is only associated with a key characteristic. For example, **page 3-135**, line 10, the header is "Cytotoxicity and degenerative cellular changes (KC #10)"; however only cytotoxicity is a key characteristic; degenerative cellular changes are associated with cytotoxicity (KC #10). Other comments:

- It is not clear in the current version how information is organized. Recommend stating the strongest evidence and highest impact first.
- **Page 3-134**, **lines 23-32, Gene and chromosomal mutation:** This section clearly states the study question (high, medium, low confidence) and overall evidence for the topic (consistent and coherent – although it is unclear if these are based on the same criteria as IARC's). This is a highly valuable outcome of a systematic review. It would be very helpful if other sections could have similar clarification of the study quality for relevant studies and the overall confidence or evaluation of a line of evidence (such as electrophilicity was presented for DNA and protein reactivity).
- **Page 3-116, Figure 3-16:** When the active form of Cr is known for a particular endpoint, it would be useful if it were indicated in the figure. It is unclear how "no evidence" (dashed line) was added to the figure; does it mean there was no direct evidence from Cr, and instead is inferred from general knowledge?
- Tables should have more discrete columns, so similar information can be compared at an easy glance. For example, Table 3-21 (beginning **page 3-148**), Cr(IV) is in the table title and, therefore, does not need to be repeated in column 1. Exposure routes and organ sites might be their own columns. Study system (e.g., human in vitro, animal in vivo, animal in vitro) might be a separate column.

3.2.4. Hepatic Effects

NIEHS Comments: The information in section 3.2.4 is clearly presented and there is an adequate level of detail for the methods and protocols. However, the use of the key characteristics is problematic when it is applied to in vitro data that is not dose contextualized to the in vivo studies. The reason for this is nearly any chemical can elicit a cell stress response and nearly every other key characteristic once a high enough dose is achieved in vitro, hence it would likely be appropriate to caveat the evidence of effects key characteristic that are derived purely from in vitro data that have not been kinetically scaled to in vivo exposures. Also, in relation to the in vitro data supporting the key characteristics findings, it is notable that supportive evidence can be derived from transformed cell lines and in certain cases there is not tissue correspondence between the cell line and the tissues affected in vivo. It is certainly understandable why these data are included; however, it would be helpful to the reader to make it clear that the in vitro system represents a general biological sensor of the effect and is not necessarily representative of target tissues, hence there is lower certainty of the in vivo relevance. Short of providing in vivo contextualization both from cell type and tissue concentration, a footnote should be added that in vitro associations with the key characteristics are potentially spurious and therefore have lower certainty.

3.2.5. Hematologic Effects

NIEHS Comments:

- **Page 3-189**, lines 2-4: Suggest rewriting the sentence beginning with "Hematology parameters routinely measured…" as "Hematology parameters, as part of a routinely measured complete blood count (CBC), are described in Table 3-27.
- **Page 3-189,** lines 4-6: Suggest modifying these two sentences to the following: "A CBC is a common blood test providing quantitative and qualitative information regarding the general health of a patient or research subject. Examples of quantitative-type information include total counts of red blood cells (RBCs), white blood cells and platelets; qualitative information, such as the RBC indices, give a morphological estimation of the RBC size and color."
- **Page 3-190, Table 3-27 (continued), Mean corpuscular cell volume row:** It is true in the days of manual microhematocrit and manual hemocytometer RBC count data collection that the MCV was a calculated value. Hematology instruments today, however, provide a direct measurement of the MCV either by electronic impedance or optical detection. The information regarding microcytosis and macrocytosis is acceptable. Also, in the first column, remove the word "corpuscular" so that the endpoint reads: "Mean cell volume (MCV).

3.2.5.3. Human Evidence

NIEHS Comments: Page 3-190, lines 3-6: The statement indicates that the hematology of these studies refer to effects on the "erythron" (circulating RBC mass). The "platelets" referred to here and described in the text below, however, would not be used as a descriptor of the erythron. Thus, if referring to changes in the erythron, delete the platelet information. Besides, the platelet information was inconsistent and adds no value to the erythron discussion. Additionally, please modify the sentence to "erythron (circulating RBC mass)".

Page 3-191, Table 3-28, first row, final column: This heading should be referred to as: "Clinical Pathology" if one or more of the following evaluations were included in the reported studies (i.e., hematology, clinical chemistry, urinalysis, or other blood measured biomarkers). If only referring to the CBC data of the reported studies, the heading could be referred to "Hematology".

3.2.6. Immune Effects

NIEHS Comments: The immune section is well presented from general topic through the human and animal evidence. The authors of this section did an excellent job in evaluating the available studies and integrating the evidence and support the NTP studies being rated as high confidence. No reference values were derived for this system. The statement and conclusions reached in section 3.2.6.4 and the evidence profile table 3-38 are supported: "*Evidence suggests that Cr(VI) may modulate the immune system through both stimulatory and suppressive actions. This conclusion is primarily based on coherent evidence of effects on ex vivo WBC function across human and animal studies, antibody responses to T cell-dependent antigen measured in animals, and reduction in host resistance to bacterial infection reported in animal studies. However, confidence in the evidence was reduced because some of the studies are low confidence and reported findings often differed across studies*."

- The evaluation of confidence/study quality of human and animal evidence streams are clearly presented; however, the evaluation of in vitro, mechanistic, or supportive immune data needs clarification in the protocol and main document in Section 2 as well as in all the subsections of 3.2.6 for immune data. This comment applies across all of the other health effects sections.
- **Page 3-229, line 15:** While the focus of this document is on oral and inhalation exposures, the addition, if available, of any evidence in the human literature on respiratory sensitization following dermal exposure or vice versa should be considered. The fact that Cr(VI) has been associated with asthma in occupational settings gives further support to the integration of evidence that Cr(VI) may modulate the immune system.

3.2.6.1 Human Evidence

NIEHS Comments: Page 3-208, lines 1 – 18: The phrasing in this section is very awkward. It appears that mitogen-stimulated T cell proliferation was elevated in workers exposed to Cr(VI) as compared to controls. It would be important to indicated the proliferative rate in unstimulated cells here to know if it is a global effect. Again, the wording is awkward — the way the B cell data are phrased suggests that LPS wasn't stimulatory, not that Cr(VI) did not have an impact on LPS-induced stimulation.

3.2.6.2. Animal Evidence

NIEHS Comments: Page 3-220, line 30: Antibody responses: While this is absolutely correct as stated, the AFC assay is more sensitive than the SRBC ELISA in nearly every NTP study that has been conducted. It is not surprising or inconsistent that there are effects in the AFC response with no effects in serum titers. NIEHS is unable to provide a reference as the NTP data are spread across multiple studies.

3.2.6.3. Mechanistic Evidence and Supporting Evidence

NIEHS Comments: Page 3-229, Hypersensitivity responses: While the focus of this document is on oral and inhalation exposures, the addition, if available, of evidence in the human literature of respiratory sensitization following dermal exposure or vice versa should be considered. The fact that Cr(VI) has been associated with asthma in occupational settings gives further support to the integration of evidence that Cr(VI) may modulate the immune system.

3.2.7. Male Reproductive Effects

3.2.8. Female Reproductive Effects

NIEHS Comments: For sections 3.2.7 and 3.2.8, the rationale describing confidence level determination relative to specific study as well as across studies is very clear (e.g. consistency/data limitations). For the most part, these sections were clearly presented. However, there is an apparent inconsistency on how summary information is presented. For example, in Table C-39 (Supplemental Material, page C-106) chemical formulae are inconsistently presented without subscripted numbers:

CRO3 should likely be CRO_3
Na2Cr2O7 should likely be $Na_2Cr_2O_7$
H2O2 should likely be H_2O_2

NIEHS recommends that EPA consider the likely precision of concentrations cited (e.g., 3.123 - 50 mM versus 3.1 - 50.0mM). Also, the word “sacrificed” is no longer commonly used; NIEHS recommends “euthanasia.”

The findings are supported, and scientific interpretations of the data are objective and reasonable.

NIEHS has no other suggestions regarding inclusion of information that could be added, or information that should be deleted.

3.2.9 Developmental Effects (Sutherland)

NIEHS Comments: The information is presented clearly, the level of detail is adequate, and the findings and scientific interpretations are appropriately synthesized, describing the strengths and limitations well. No recommendation for additional information that should be added or deleted, no scientific criticisms on this work, and the evaluation that the evidence suggests that Cr(VI) likely causes developmental effects in humans under relevant exposure circumstances is supported.

The in vivo data are carefully evaluated and given levels of confidence, the discussion is well done, and the strengths/limitations of each study are addressed. This level of assessment or evaluation is not done for the in vitro and mechanistic data (per protocol description). At this time, criteria may not be established for each of the techniques used; however, a level of confidence in the in vitro data (once criteria are established) would strengthen these assessments.

- **Page 3-300**, line 4: The word "that" is repeated twice in the sentence. "Several in vitro and in vivo studies identified mechanisms that that are potentially…"

4. Dose-Response Analysis

4.1. Oral Reference Dose for Effects Other Than Cancer

NIEHS Comments: The information in section 4.1 is clearly presented and there is an adequate level of detail for the methods and protocols. However, the use of the key characteristics is problematic when it is applied to in vitro data that is not dose contextualized to the in vivo studies. The reason for this is that nearly any chemical can elicit a cell stress response and nearly every other key characteristic once a high enough dose is achieved in vitro, hence it would be appropriate to caveat the evidence of effects key characteristic that are purely derived from in vitro data that has not been kinetically scaled to in vivo exposures. Also, in relation to the in vitro data supporting the key characteristics findings, it is notable that supportive evidence can be derived from transformed cell lines and in certain cases there is not tissue correspondence between the cell line and the tissues effected in vivo. It is certainly understandable why these data are included; however, it would be helpful to the reader to make it clear that the in vitro system represents a general biological sensor of the effect and is not necessarily representative of target tissues, hence there is lower certainty of the in vivo relevance. Short of providing in vivo contextualization both from cell type and tissue concentration, a footnote should be added that in vitro associations with the key characteristics are potentially spurious and, therefore, have lower certainty.

4.2. Inhalation Reference Concentration for Effects Other than Cancer

NIEHS Comments: Section 4.2 is clearly described, the approach and choices are reasonable, and the conclusions are supported by the evidence.