SCIENCE ADVISORY BOARD

A Federal Advisory Committee to the U.S. Environmental Protection Agency

September 27, 2023

EPA-SAB-23-009

The Honorable Michael S. Regan
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: Transmittal of the Science Advisory Board Report titled, "Review of EPA's draft IRIS Toxicological Review of Hexavalent Chromium"

Dear Administrator Regan,

Please find enclosed the final report from the Science Advisory Board (SAB) titled, *Review of EPA's draft IRIS Toxicological Review of Hexavalent Chromium*. The EPA's Office of Research and Development, Integrated Risk Information System (IRIS) program developed their draft Toxicological Review of Hexavalent Chromium and requested that the SAB review and provide comments regarding the scientific soundness of the conclusions presented in the IRIS document.

In response to the EPA's request, the SAB identified subject matter experts to augment the SAB Chemical Assessment Advisory Committee (CAAC) and assembled the SAB Hexavalent Chromium (Cr(VI)) Review Panel to conduct the review. The SAB Cr(VI) Review Panel met virtually on February 15, 2023 to hear a presentation by EPA staff, and then at an in-person meeting on March 29-31, 2023 to deliberate on the agency's charge questions. Another virtual meeting was held on July 19 and 27, 2023 to discuss the panel's draft report. Oral and written public comments were considered throughout the advisory process. This report conveys the consensus advice of the SAB along with detailed comments on specific issues provided in two appendices. Appendix A contains recommended changes that are primarily editorial in nature and Appendix B contains comments from individuals, that are either not reflective of the consensus view or provide supplementary information, and are included for the sake of completeness of this report.

In general, the SAB agreed with many of the conclusions presented in the draft IRIS document. The SAB also identified many areas that would benefit from further clarification to enhance transparency and increase the utility of the IRIS document. The SAB provided numerous recommendations and would like to highlight the following ones, with additional details described within the full report. The SAB recommends that EPA consider the following points as

they revise their documents:

While the literature search strategy was adequately described, the SAB provided multiple suggestions to enhance clarity. In general, the SAB agreed that EPA's conclusions relative to non-cancer hazard identification were scientifically supported; however, in many cases greater clarity and transparency would strengthen the draft IRIS assessment. Specifically, the EPA should provide a more robust discussion of the scientific justifications for the selection of the critical study for the respiratory hazards (both nasal and non-nasal) and consider if studies other than Glaser *et al.* (1990) or Gibb *et al.* (2000a) better support the determination of points of departure (POD) for non-cancer respiratory hazards; and the selection of Alanine transaminase (ALT) data for the derivation of the POD for hepatic effects.

The SAB noted that absorption efficiency would be expected to be non-linear relative to luminal Cr(VI) concentration which EPA should take into consideration as they explore nonlinear low dose extrapolations for both cancer and non-cancer effects. Specifically, the SAB recommends that the EPA consider using toxicokinetic factors (i.e., dose-dependency in chromium accumulation) in low dose extrapolation for the oral route of exposure.

The SAB agreed that Cr(VI) likely causes gastrointestinal cancer. A strong majority (12 of 14 panel members) concurred with the EPA decision to use a mutagenic mode of action to assess risks, whereas 2 of 14 panel members recommended the assessment be based on a regenerative hyperplasia, threshold approach. Irrespective of the low dose extrapolation approach, as noted above, the potentially reduced absorption efficiencies at low doses should be considered relative to low dose extrapolation of cancer risks from the point of departure.

As the EPA finalizes the draft IRIS Toxicological Review of Hexavalent Chromium [Cr(VI)], the SAB encourages the EPA to address the SAB's concerns raised in the enclosed report and consider their advice and recommendations. The SAB appreciates this opportunity to review EPA's draft IRIS document and looks forward to the EPA's response to these recommendations.

Sincerely,

/s/

Alison C. Cullen, Sc.D.
Chair
EPA Science Advisory Board

/s/

John Morris, Ph.D.
Chair
EPA SAB Cr(VI) Review Panel

Enclosure

NOTICE

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The SAB is a chartered federal advisory committee, operating under the Federal Advisory Committee Act (FACA; 5 U.S. Code 10). The committee provides advice to the Administrator of the U.S. Environmental Protection Agency on the scientific and technical underpinnings of the EPA's decisions. The findings and recommendations of the Committee do not represent the views of the Agency, and this document does not represent information approved or disseminated by EPA.

U.S. Environmental Protection Agency
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SAB Review of EPA's draft IRIS
Toxicological Review of Hexavalent Chromium

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ACRONYMS AND ABBREVIATIONS

ADAF	Age-dependent Adjustment Factor
AIC	Akaike's information criterion
ALT	Alanine transaminase
AOP	Adverse Outcome Pathway
AST	Aspartate transaminase
BMD	Benchmark Dose
BMDL	Benchmark Dose lower bound limit
BMR	Benchmark Response
BW	Body Weight
Cr	Chromium
Cr(III)	Trivalent Chromium
Cr(VI)	Hexavalent Chromium
CSF	Cancer Slope Factor
FVC	Forced Vital Capacity
GI	Gastrointestinal
HAWC	Health Assessment Workspace Collaborative
HEC	Human Equivalent Concentration
HED	Human Equivalent Dose
HERO	Health and Environmental Research Online
IRIS	Integrated Risk Information System
LOAEL	Lowest Observed Adverse Effect Level
MOA	Mode of Action/Mechanism of Action
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
OSF	Oral Slope Factor
osRfD	organ/system-specific RfD
osRfC	organ/system-specific RfC
PBPK	Physiologically-based pharmacokinetic
PECO	Population (including animal species), Exposure, Comparator, and Outcomes
PK	Pharmacokinetic
POD	Point of Departure
$POD_{[HEC]}$	Human Equivalent Concentration POD
$POD_{[HED]}$	Human Equivalent Dose POD
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red Blood Cell
RfD	Reference Dose
RfC	Reference Concentration
UF	Uncertainty Factor
UF_A	animal-to-human uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has developed a draft *Toxicological Review of Hexavalent Chromium* in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is produced and maintained by EPA's Center for Public Health and Environmental Assessment within the Office of Research and Development (ORD). Draft IRIS assessments contain information about chemicals that encompass hazard identification and a dose-response assessment, two of the four steps in the human health risk assessment process. When used by risk managers in combination with information on human exposure and other considerations, draft IRIS assessments support the Agency's regulatory activities and decisions to protect public health.

The assessment under review updated a previous draft IRIS assessment of hexavalent chromium [Cr(VI)] (posted in 1998) that included an oral reference dose (RfD) and inhalation reference concentration (RfC) for effects other than cancer, a determination of carcinogenic potential, and inhalation unit risk (IUR) for carcinogenic effects. The updated draft Toxicological Review of Cr(VI) includes a review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to Cr(VI). The systematic review protocol for Cr(VI) and appendices for toxicokinetic information, dose-response modeling, and other supporting materials were provided as *Supplemental Information—Appendix A: Systematic Review Protocol for the Hexavalent Chromium draft IRIS assessment* and *Supplemental Information—Appendices B, C, D, and E* to the draft Toxicological Review.

The EPA's Office of Research and Development requested that the SAB conduct a scientific peer review of EPA's draft IRIS assessment. In response to the EPA's request, the SAB identified subject matter experts to augment the Science Advisory Board (SAB) Chemical Assessment Advisory Committee (CAAC) and assembled the SAB Hexavalent Chromium (Cr(VI)) Review Panel to conduct the peer review. The SAB Cr(VI) Review Panel met virtually on February 15, 2023 to hear a presentation by EPA staff, and then at an in-person meeting on March 29-31, 2023 to deliberate on the agency's charge questions. Another virtual meeting was held on July 19 and 27, 2023 to discuss the panel's draft report. Consideration of oral and written public comments was encouraged throughout the advisory process.

The panel identified numerous instances in which the analyses and conclusions in EPA's draft IRIS assessment could be revised to be more thorough and transparent. This report is organized by the charge questions raised by the agency and are followed by the consensus response and recommendations. Additional information and minority opinions are presented in Appendices at the end of the report. The panel provided key recommendations that are necessary to improve the critical scientific concepts, issues, and/or narrative within the EPA's draft IRIS assessment. The panel deemed these recommendations (Tier 1) as important for improving the transparency of the agency's conclusions and to bolster the supporting evidence for them. Tier 2 recommendations are included for EPA to consider as they revise their assessment, and Tier 3 recommendations represent suggestions to inform future reviews or research efforts.

A list of acronyms and abbreviations can be found at the front of this report to assist in orienting the reader to the terminology used throughout the panel's responses to the Charge Questions. Comments that are primarily editorial in nature are presented in Appendix A. Additional supplementary comments and minority opinions are presented in Appendix B. All materials and comments related to this report are available at:
https://sab.epa.gov/ords/sab/f?p=114:18:15517509861657:::RP,18:P18_ID:2618

RESPONSE TO CHARGE QUESTIONS

Charge Question #1 - Study Identification and Inclusion

The Toxicological Review describes and applies a systematic review process for identifying and screening pertinent studies that is described in detail in Section 1.2.1 (Literature Search and Screening) and Appendix A (Systematic Review Protocol). Please comment on whether the literature search strategy and screening criteria for Cr(VI) are appropriate and clearly described. Please identify additional peer-reviewed studies of Cr(VI) compounds that the assessment should consider[1].

Overall Comments

In general, the literature search strategy is clearly described. Overall, the panel considered EPA's approach for gathering all the information in the draft IRIS assessment to be rigorous. The relationship between the updates listed in Chapter 12 of the October 2022 Systematic Review Protocol (Protocol) and the October 2022 External Review Draft assessment (draft assessment) is not identified. For example, it is not clear if the revisions in the Protocol were added in time to be adequately considered in the October 2022 draft assessment.

In general, the screening criteria are appropriate and clearly described. However, the panel identified a number of areas where the bases for EPA's choices of scientific literature were not transparent, and clarifications are recommended. For example, there is some lack of transparency and clarity regarding the decisions to include and exclude certain literature. This makes it more difficult for external reviewers to replicate EPA's strategy, and to fully understand why some studies were not cited as important especially regarding mode of action (MOA) and mechanisms. For example, in the flow chart of the literature search strategy, exclusions after the review of abstracts or the full text are lumped together without categorization by reason for exclusion. In a systematic review, at least when using tools such as *Covidence*, there are two steps for screening and exclusion: one based on abstract review, and one based on full text review. For the latter where a more careful review of potentially relevant literature is needed, specific reasons would be provided for exclusion. This list of reasons does not have to be exhaustive, but it would help make the rationale more transparent. This is especially important for manuscripts from the Costa laboratory (see Appendix B of this report where 46 citations are provided), which should be considered in the IRIS assessment since they illustrate several unique toxic effects of Cr(VI) and Cr(III).

The majority of the excluded studies are listed in the Cr(VI) Health and Environmental Research Online (HERO) database. Some of these (mainly from the Costa laboratory) are especially relevant to Cr(VI) (and CrIII) genotoxicity *in vivo* and *in vitro*, across species, in

[1]Newly identified studies (i.e., studies identified by EPA or the public that meet PECO criteria but were not addressed in the external review draft, for example due to recent publication) will be characterized by EPA and presented to the peer review panel. This characterization will focus on EPA's judgment of whether the studies would have a material impact on the conclusions (i.e., identified hazards or toxicity values) in the external review draft. The peer review panel is asked to review EPA's characterization and provide tiered recommendations to EPA regarding which studies, if any, to incorporate into the assessment before finalizing.

different target organs and for use in biomonitoring. Others are relevant to evidence for a nonlinear dose-response for oral Cr(VI) due either to toxicokinetic or toxicodynamic factors and a number are from the ToxStrategies group or Haney and co-workers. Others are relevant to human studies.

The panel recognizes that the Cr(VI) literature is vast, and studies logged into HERO and/or Health Assessment Workspace Collaborative (HAWC) databases may not necessarily be cited in the final IRIS assessment if they did not meaningfully contribute or alter the conclusions made by the EPA. Other excluded studies, not specific to Cr(VI), pertain to MOA frameworks, and are not found in the Cr(VI) HERO database. Two panel members suggested that these framework publications could provide guidance for a comparative evaluation of tumors from oral exposure to Cr(VI), considering the two modes of action (MOAs) [non threshold-mutagenicity and threshold-cytotoxicity-induced regenerative hyperplasia in the small intestine of the mouse]. These studies, not specific to Cr(VI), are included in Appendix A of this report.

Recent publications regarding drinking water criteria need to be identified and added to Table B-3 in the Supplemental Materials. A reasonable starting point could be the table of toxicity and regulatory criteria presented at the February 15th meeting, with confirmation of its accuracy, as a source for updating Table B-3. Tables 7 through 9 of the Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (USEPA, 2022) show that the number of "supplemental studies" far outnumber the number of studies included as part of the Population (including animal species), Exposure, Comparator, and Outcomes (PECO) strategy. Supplemental studies did not receive the same level of scrutiny (i.e., considering confidence when interpreting this literature) as the studies tallied in Tables 7 and 8, despite their potential importance for MOA considerations in the overall risk assessment.

Recommendations

Tier2

- The EPA should provide more clarity and transparency relative to the criteria for inclusion and exclusion of specific studies, and tabulate the excluded papers (especially those listed below under "Other Studies for inclusion" section) in an appendix so that the reader of the draft IRIS assessment can understand the types of studies that were eliminated from consideration and why.
- The EPA should clarify that not all studies in the HERO and HAWC databases are cited in the report, and that the focus was on citing the subset of the literature that drove EPA's conclusions.
- The EPA should add publications in supplemental materials regarding non-Cr(VI) MOA frameworks and drinking water criteria.
- The EPA should provide more clarity and transparency relative to the basis for the differing degree of scrutiny applied to the supporting studies.
- Figure 1. Literature search flow diagram for Cr(VI) (p. 27 of the Systematic Review Protocol for the Hexavalent Chromium Assessment (USEPA, 2022) draft IRIS assessment) presents a nice flow chart according to *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) guidelines; but the "potentially

relevant" categories don't seem to align with the categories in Table 6 of the Systematic Review Protocol for the Hexavalent Chromium Assessment (USEPA, 2022).

- EPA should present the health effects studies in Figure 1 categorized by health outcome (i.e., include information from Tables 7 and 8 in Figure 1 of the Systematic Review Protocol for the Hexavalent Chromium Assessment (USEPA, 2022)).
- Table 12 of the Systematic Review Protocol for the Hexavalent Chromium Assessment (USEPA, 2022)is very informative as a summary of key elements to be considered in epidemiological studies, but there is no similar table for animal studies.
- EPA should consider adding a table with key elements to be considered for animal studies.

Charge Question #2 - Study Evaluation

The Toxicological Review describes the results of the evaluations of individual studies in Section 2.2 (Study Evaluation Results) and presents and analyzes the findings from those studies deemed informative in the relevant health effect-specific synthesis sections.

a. Results from individual Cr(VI) studies are presented and synthesized in the health system-specific sections. Please comment on whether the presentation and analysis of study results is clear, appropriate, and effective to allow for scientifically supported syntheses of the findings across sets of studies.

The panel found that the sections in the draft IRIS assessment, where the analysis of studies was presented, were generally clear and effective. However, several areas requiring further clarification are explained in the recommendations below.

b. Please comment on whether the study confidence conclusions for the Cr(VI) studies are scientifically justified, giving appropriate consideration to important methodological features of the assessed outcomes. Please specify any study confidence conclusions that are not justified and explain any alternative study evaluation decisions.

As described in Section 6.1 of the draft IRIS assessment, each health outcome in a study is rated on a number of evaluation domains (e.g., reporting quality, observational bias/blinding, exposure method sensitivity). The overall confidence rating for each outcome in a study considers the ratings for the evaluation domains and the likely impact of the deficiencies that were identified related to bias, lack of sensitivity, or inadequate reporting on the results. Overall, the study confidence conclusions appear scientifically justified and clearly described, considering important study attributes such as methodological features of the assessed outcomes.

The study confidence conclusions for epidemiological studies are not always consistent when deficiencies in the exposure or health outcome domains are noted. Sometimes studies with such limitations are included in the review and assigned low confidence (e.g., p. 3-63), whereas on

other occasions studies are excluded because of such deficiencies (e.g., p. 3-283).

Recommendations:

Tier 2:

- The EPA should review their selection and discussion of epidemiologic studies for consistency in approach when significant deficiencies in exposure or health outcome assessment are noted. The selection of epidemiologic studies should maintain similar standards for different health outcomes to remove inconsistency. The designation of "low confidence" or "uninformative" needs to be more specific in the criteria used.
- For increased transparency and ease of reference, the EPA should consider adding the figure summaries to the main draft IRIS assessment in addition to appearing in HAWC, which is more difficult to navigate than the draft IRIS assessment itself.

Charge Question #3 - Noncancer Hazard Identification and Toxicity Value Derivation

For each health effect considered in the assessment and outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations and to support the conclusions presented. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified, and appropriately consider health effects in susceptible subpopulations or lifestages (e.g., children) to the extent possible, given the available data. In addition, please separately comment on whether the dose-response decisions are transparent and scientifically justified, including: study selection for dose-response analyses; point of departure (POD) estimates, including modeling choices and assumptions, and dosimetric adjustments; selection of uncertainty factors and derivation of candidate values; selection of organ/system-specific RfDs/RfCs; and confidence in the calculated values.

Overall Comments

In general, the available information on the non-cancer effects is clearly and appropriately synthesized, including strengths and limitations to support the conclusions for each health effect presented in the draft IRIS assessment. The panel agreed that, in general, the weight of evidence decisions for hazard identification for each of the non-cancer health effects is clearly described and justified, and that health effects in susceptible subpopulations are appropriately considered.

In considering the basis for the decision to use a No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL), rather than a Benchmark Dose lower bound limit (BMDL), as the point of departure (POD) for several endpoints, the panel agreed that the use of a NOAEL or LOAEL for these endpoints, in general, is appropriate and consistent with EPA Benchmark Dose (BMD) modeling guidance.

Please note that for some of the organ-specific effects, one or two of the panel members raised concerns that were not shared by the majority of the other panel members. Accordingly, the general concern is noted and more detailed comments by the panel member(s) are included in Appendix B.

Recommendations:

Tier 2:

- While the panel agreed with the decisions outlined in the draft IRIS assessment, the panel suggests that the EPA should mention in discussing the development of the Reference Dose (RfD, p. 4-25, Section 4.1.8) that the present value is within approximately 3-fold of the previous RfD developed in 1998.

a. Gastrointestinal (noncancer):

i. *The **evidence indicates** that oral exposure to Cr(VI) likely causes GI tract toxicity in humans given sufficient exposure conditions[2]. This conclusion is primarily based on robust studies in rodents that found Cr(VI) causes non-neoplastic effects in the GI tract.*

The panel agreed with the conclusion that oral exposure to Cr(VI) likely causes gastrointestinal (GI) tract toxicity in humans given sufficient exposure conditions. The choice of the final RfD that was based on diffuse epithelial hyperplasia in the female mouse small intestine was appropriate.

Further, the panel agreed that the available data on gastrointestinal effects (noncancer) were clearly and appropriately synthesized to describe the strengths and limitations. Taken together, the weight of the available scientific information presented in the draft IRIS assessment reasonably supports the conclusion that with sufficiently high exposure to Cr(VI) over a sufficiently long duration (i.e., "given sufficient exposure conditions" as stated in Table 3-10), it is likely that Cr(VI) causes gastrointestinal effects (noncancer) in the general human population, which includes potentially susceptible subpopulations. The bases for this weight-of-evidence decision are clearly described in the text (e.g., *Integration of Evidence* on pp. 3-57 and 3-58) and Table 3-10 of the draft IRIS assessment.

ii. *A POD from NTP (2008), a 2-year drinking water bioassay in rodents, was selected to calculate an organ/system-specific RfD of 9×10^{-4} mg/kg-d based on diffuse epithelial hyperplasia in the female mouse small intestine. A composite uncertainty factor of 100 was used to account for intraspecies, animal-to-human, and LOAEL-to-NOAEL uncertainties. This organ/system-specific RfD (osRfD) was selected as the overall RfD. Please comment on whether the selection of the overall RfD is scientifically justified and clearly described.*

[2] As described in the Toxicological Review, the exposure conditions for each identified hazard are further defined through dose-response analyses.

The majority of the panel agreed that the selections of the POD and composite uncertainty factor and osRfD were scientifically justified. The RfD was derived using state of practice approaches.

Most of the panel agreed with EPA that the uncertainties associated with the BMD modeling (including the alternative approaches) discussed in Sections 4.1.2.2. and Appendix D.1.1.1 are too large for use of a BMDL as the POD. Most panel members also agreed with EPA that the choice of the LOAEL as the POD is appropriate and consistent with EPA BMD modeling guidance. As discussed, in Section D.1.1.1, the LOAEL divided by a UF of 10 for LOAEL-to NOAEL extrapolation is within the range of the BMDLs that were developed. However, one panel member expressed concerns about the scientific justification for the overall RfD. More detailed comments regarding these concerns are provided in Appendix B.

iii. *EPA determined that the dataset for diffuse epithelial hyperplasia of the duodenum in female mice from NTP (2008) was not amenable to BMD modeling because uncertainty in estimating the BMD is too high. As a result, the LOAEL was used as the POD for toxicity value derivation of this endpoint in female mice. Female mouse hyperplasia was selected as the osRfD for gastrointestinal toxicity because females may be the more sensitive group. However, alternative approaches are presented and weighed in the toxicological review. Please comment specifically on whether the data and modeling decisions for the osRfD for gastrointestinal tract toxicity are scientifically justified and clearly described.*

The EPA determined that the dataset for diffuse epithelial hyperplasia of the duodenum in female mice from NTP (2008) was not amenable to BMD modeling because the uncertainty in estimating the BMD is too high. As a result, the LOAEL was used as the POD for toxicity value derivation of this endpoint in female mice. Female mouse hyperplasia was selected as the osRfD for gastrointestinal toxicity because females may be the more sensitive group. Additionally, alternative approaches are presented and weighed in the draft IRIS assessment. The panel deemed the data and modeling decisions for the osRfD for gastrointestinal tract toxicity to be scientifically justified and clearly described.

Regarding the choice of using the LOAEL for deriving the POD and the modeling approach used by the EPA, one panel member expressed concerns that are detailed in Appendix B.

Recommendations:

Tier 2:

- The EPA should consider for inclusion as another candidate for RfD determination using the Thompson *et al.*, (2012; 2011) studies for BMD modeling. The EPA did not use these data (Oral RfD, GI tract pathology (Table 4-1)) because "these studies used smaller sample sizes and shorter exposure duration" even though it was acknowledged that the range of exposures was wider in the Thompson *et al.*, (2011; 2012) studies than in the studies used for BMD modeling. The Thompson *et al.* studies were commensurate with a recommended 90-day study design and, in fact, examined not only a wider range of doses, but also a greater number of doses. BMD modeling should be performed on the

90-day studies (both NTP, 2007 and the Thompson *et al.* data 2011, 2012) and an additional sub-chronic to chronic UF should be applied.

- The EPA should consider performing dose-response modeling for gastrointestinal effects in the rat. While it would be helpful in characterizing the uncertainty associated with animal model selection, as a different and potentially equally relevant animal model, no GI effect POD_{HED} comparisons are possible for the rat because none of the GI tract effects in Table 3-50 (p. 3-314) were carried forward for dose-response modeling (see Tables 4-3, 4-4, D-1, D-2, D-3).
- The EPA should consider the minimally adverse status of the noncancer GI effects in selecting the Monte Carlo Pharmacokinetic (PK) analysis percentile, as it was in the choice of a benchmark response (BMR) of 10% versus a lower value (p. 4-10, lines 7-7). To account for interindividual variability, the Human Equivalent Dose (HED) was determined by Monte Carlo analysis using the lower 1% value of 20,000 Monte Carlo PK simulations needed to achieve the internal dose POD (i.e., 0.0911 mg/kg-d in Figure D-9 on p. D-47).

b. Respiratory (noncancer outside of nasal cavity):

*i. The **evidence indicates** that inhalation exposure to Cr(VI) likely causes lower respiratory tract effects in humans given sufficient exposure conditions. This conclusion is primarily based on inflammatory effects indicative of lung injury in medium confidence animal studies, supported by observations of decreased lung function among chromium exposed workers in low confidence human studies and mechanistic observations that support the biological plausibility of an inflammatory tissue response following Cr(VI) exposure that is interpreted to lead to impaired function or adverse structural changes. Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed that the available data on respiratory effects (noncancer outside of nasal cavity) are clearly and appropriately synthesized to describe the strengths and limitations. EPA concluded (p. 3-38, lines 18-19) that "overall, the available evidence indicates that Cr(VI) likely causes lower respiratory tract effects in humans." Table 3-7 of the draft IRIS assessment (p. 3-41 to 3-45) includes the evidence profile table for these respiratory effects, which among other information contains factors that both increase certainty (e.g., consistent evidence of some inflammatory changes in two medium confidence studies in two rat strains) and decrease certainty (e.g., lack of duration-dependence for some effects weakened with longer exposures) along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary conclusion.

The text of the draft IRIS assessment (Section 3.2.1) also contains information that is relevant to support the weight-of-evidence for respiratory effects (noncancer outside of nasal cavity). Taken together, the weight of the available scientific information presented reasonably supports the conclusion that with sufficiently high exposure to Cr(VI) over a sufficiently long duration (i.e., "given sufficient exposure conditions" as stated in Table 3-7), it is likely that Cr(VI) causes respiratory effects (noncancer outside of nasal cavity) in the general human population, which includes potentially susceptible subpopulations.

ii. *A POD from Glaser et al. (1990), a 90-day inhalation bioassay in rodents, was selected to calculate an osRfC of 1 × 10^{-4} mg/m^3 based on histopathological/cellular responses in the lung. For most endpoints that served as the basis for this osRfC, a composite uncertainty factor of 1,000 was used to account for intraspecies, animal-to-human, subchronic-to-chronic, LOAEL-to-NOAEL, and database deficiency uncertainties. Please comment on whether the selection of the POD is scientifically justified and clearly described.*

The panel agreed with the conclusion that Cr(VI) likely causes lower respiratory tract effects is appropriate. Data from human, animal toxicity and mechanistic studies all support the conclusion that Cr(VI) can cause pulmonary effects. However, some members of the panel did not agree that the available data have been clearly described or were appropriately synthesized. In many areas the text is too generalized, consisting merely of a reporting of the quantitative results without any interpretation relative to their magnitude or health-related significance or comprehensive analysis. All of these concerns serve to significantly diminish the quality of the draft IRIS assessment relative to effectively supporting its scientific syntheses, interpretations, and conclusions. The draft IRIS assessment could be strengthened by providing more robust and effective support of its scientific syntheses, interpretations, and conclusions.

The panel agreed that a major weakness of the draft IRIS assessment centers on the selection of Glaser *et al.* (1990) as the critical study for determining a POD. The panel noted multiple concerns related to this study and its evaluation (Section 3.2.1.2). The Glaser *et al.*, 1990 study appears to be a chapter published in a book, and therefore, it is unclear whether it was scientifically peer reviewed. Glaser *et al.* (1990) provides absolutely no description of the statistical procedures and does not indicate what the confidence intervals in figures/tables represent. This issue is of particular concern because their earlier work (Glaser *et al.*, 1985) relied on multiple t-tests for statistical analysis. Therefore, statistical conclusions in Glaser *et al.*, (1990) study cannot be relied upon. Although the brief text of Glaser *et al.* (1990) states that broncho-alveolar hyperplasia and histiocytosis were observed, not a single photomicrograph was provided to assess or characterize these lesions. EPA's designation of this publication as a medium confidence study is unwarranted and should be clearly justified on the basis of current EPA protocols for study evaluation or the selection of the critical study should be revised.

There are multiple instances in the text of the draft IRIS assessment where essential detail is lacking relative to particle size and characteristics. First, sodium chromate or dichromate salt aerosols are described as hygroscopic or "strongly" hygroscopic. Hygroscopicity is an important property, particularly with respect to regional aerosol deposition patterns. Yet, based on the current description included in the draft IRIS assessment, this property is apparently not taken into consideration relative to regional deposition of these aerosols. Often particle size information of individual studies is not provided in the main text of the draft IRIS assessment. Determination of particle size is an absolutely essential methodological feature of inhalation toxicity evaluations. The reader should not have to dig deep into the draft IRIS assessment (or other sources) to discover the particle sizes. The concern relative to information on particle size is particularly true for evaluation of the epidemiological studies. Because particle sizes may well differ in various occupational settings due to the differing conditions in which the particles were

generated, particle sizes from one occupation are not necessarily reflective of those in other occupations or in the general environment. If information exists on the particle sizes of chromium aerosols in the general environment, this should be explicitly provided.

The panel agreed that the evaluation of respiratory tract responses should be strengthened. A significant and repeated source of confusion centers around the description of generalized response patterns to insoluble dusts to describe/evaluate the effects of highly soluble Cr(VI) aerosols. This greatly diminishes the clarity and transparency of the analysis. The magnitude of any chromate-associated change in health parameters should be discussed to improve clarity and transparency. A small change in pulmonary lavage parameters in animal studies or a small change in pulmonary function should be characterized as such. A comprehensive comparison of animal and human data (e.g., pulmonary function changes suggestive of restrictive disease and the observation of fibrotic changes in animals) would strengthen the scientific basis of any conclusions. In obligate nose breathing rodents, observation of peculiar breathing sounds and/or obstructive respiratory dyspnea are the hallmark of nasal obstruction and should be discussed in light of this fact. Finally, pulmonary function studies performed prior to the promulgation of the 1987 American Thoracic Society guidelines may in fact provide useful data; exclusion of all studies prior to 1987 appears to be arbitrary and is not scientifically supported.

The EPA should clarify the discussion of pulmonary chromium particle deposition. The text of the draft IRIS assessment (e.g., p. 3-11, p. 3-39) states that 5 µm will typically deposit proximal to the trachea, 2.5-5 µm will generally deposit in the tracheobronchial airways, and particles less than 2.5 µm generally deposit in the pulmonary region. Yet, the fractional deposition of particles of 2.5-5 µm diameter is actually twice as high in the alveolar than tracheobronchial region (see for example, Figure 5 or 8 in the Burleson and Schlesinger, 2015, pp. 511-536).

The EPA should clarify the inconsistencies in the presentation of some studies on human lower respiratory effects of Cr(VI). Based on the literature summary table in HAWC (cited on p. 3-20 in the draft IRIS assessment), the confidence assignments for the literature studies seem generally acceptable. However, the evaluation of Sobaszek *et al.* (1998) is quite confusing. Multiple studies were excluded because of deficiencies in exposure measures; yet Sobaszek *et al.* (1998) was not excluded even though Table 3-4 states it had "no quantitative exposure measures." The exposure to Cr(VI) was inferred based solely on occupation in this study. Other studies by Huvinen *et al.* (2002a, 2002b) were deemed critically deficient because of the same deficiency where exposure was assessed based on occupation.

The discussion of altered pulmonary function in chromium workers (p. 3-22) would benefit from a more thorough analysis. The EPA should characterize the magnitude of the observed changes. Specifically, whether the observed changes are considered to be mild vs. severe, etc., and whether they suggest a specific type of lung disease (e.g., obstructive, restrictive). This characterization would place these findings in an appropriate health context. It is interesting that the study by Li *et al.*, (2015) reported decrements in forced vital capacity (FVC) but an actual increase in the ratio of the forced expiratory volume in the first one second to the forced vital capacity of the lungs (FEV1/FVC). There was also a reported increase in maximal expiratory flow (MEF) (p. 3-24). These observations have specific implications relative to the type of pulmonary change (e.g., restrictive vs. obstructive), particularly because fibrotic lesions were

observed in the rodent inhalation toxicity studies. The draft IRIS assessment does not provide this type of comprehensive analysis, but it should. The characterization of FVC at 81% of predicted in the control population misses a critical point. This percent of predicted value is typically considered to be at the lower end of normal; the EPA should acknowledge and discuss this issue.

Overall, the EPA should provide a more careful analysis in the bronchoalveolar lavage (BAL) response section of the draft IRIS assessment. The text could be strengthened by an analysis of the magnitude of the responses (mild, moderate, etc.). Some of the reported responses appear to be quite mild; the reader is left unaware of the EPA evaluation of such responses. Third, a stronger comparison among the studies is warranted. An increased bronchoalveolar lavage (BAL) neutrophil response is fundamentally different toxicologically than an increase in macrophages. This should be acknowledged and discussed. As an editorial note, some portions of the text use the acronym BAL while others use BALF (bronchoalveolar lavage fluid). Consistency would enhance clarity.

The EPA should correct and more carefully present the description of the macrophage accumulation data from the Glaser *et al.* (1990) study. The study is apparently misquoted in the description of macrophage accumulation following sodium dichromate inhalation on p. 3-30 (lines 12-14). The finding of alveolar and peribronchial region accumulation of macrophages refers to the authors' previous 1-year duration study, not to the 90-day Glaser *et al.* (1990) study. The only comment about the 90-day response in this study is, "Histopathology of the upper airways revealed focal inflammation but very seldom hyperplasia."

In describing the lung weight responses on p. 3-32, the EPA could more clearly indicate whether the studies measured lung wet (total) weight or dry weight. The Glaser *et al.* (1990) study reported dry weight. The interpretation of dry weight response is quite different than wet (total) weight response.

In the *Other Findings* section (p. 3-33) of the draft IRIS assessment, the EPA could note as a study deficiency that none of the animal studies included examination of nasal responses. This lack of description is somewhat surprising because the effects of chromium on the nose were well known, and by the time these studies were performed, standardized approaches for examining rodent nasal responses were established.

The EPA needs to enhance the section that describes the regional particle deposition versus particle size (p. 3-39). This section is overly simplistic and does not reflect the current state of the art (see previous comments). The rodent study used the highly hygroscopic sodium dichromate. The particles most likely grew considerably in size during inspiration, which needs to be explicitly acknowledged. The concept that workers are exposed to larger particles than those in the rodent studies needs more justification/explanation. The text only cites one reference (Kuo *et al.*, 1974) and that study only focused on a single occupation – electroplating. Vastly different particle sizes would be anticipated in other occupations (e.g., welding, roasting for chromate production). The text of Kuo *et al.* (1974) also indicates that in some personal sampling, sample particle size was 0.75 µm. This manuscript also cites Bonin *et al.* (1995) which indicates that particles as small as 0.3 µm can be observed in chromium plating processes. Also

relevant to this issue is that workers may well be mouth breathing during the workday, which would greatly increase penetration of inspired aerosols to the lower respiratory tract. Much greater clarity is required in the IRIS assessment.

The EPA should correct and clarify some reporting issues in the paragraph on modeling (p. 4-38, lines 9-22 of the draft IRIS assessment). This paragraph seems to indicate that modeling efforts were made over concentrations of 1-136 mg/m^3 and at 54 mg/m^3 yet the animal exposure concentrations were 1000-times lower ($\mu g/m^3$). Presumably this is a typo, but this needs to be checked. It states that a density of sodium dichromate of 2.52 g/cm^3 was assumed, but this is wrong. The sodium dichromate was generated by nebulization of an aqueous solution, and sodium dichromate is hygroscopic. While 2.52 g/cm^3 may be the density of crystalline sodium dichromate it certainly is not reflective of the density of the aerosol the animals inhaled.

Recommendations:

Tier 1:

- The EPA should reevaluate the analysis and potential use of the Glaser *et al.* (1990) study for POD determination as discussed above. At the very least, the use of this study to derive a POD for pulmonary effects requires considerably more justification and qualification. If the EPA is aware of additional information on the Glaser *et al.*, 1990 data this should be clearly stated in the IRIS assessment.
- The EPA should directly address the issue of particle sizes in chromate aerosol exposures. Particle sizes should be explicitly stated for each study that is cited, or if the particle size is not known, this should be so stated. This applies to both experimental animal studies and to occupational epidemiological studies. That chromate aerosols are hygroscopic needs to be explicitly considered in evaluation of each study. Particle sizes should not be assumed to be similar in all occupational settings. Speculation on differing particle sizes in the workplace or general environment should be clearly indicated as such.
- The EPA should provide a more comprehensive analysis of the animal and human response data. Health data from human and animal studies need to be more carefully described and interpreted relative to the absolute magnitude of any observed effects, the similarity/differences between animal and human responses, and the health significance of clinical observations such as labored breathing in rodents.
- The EPA should not exclude pulmonary function studies performed prior to 1987, since it is not scientifically justified.

Tier 2:

- The EPA should modify Table 3-7 to include an explanation for why Cr(VI) is judged to "likely cause(s)" the effects at issue, as provided in Table 3-10.
- The EPA should reconsider the database uncertainty factor (UF_D) of 3. The total UF of 1,000 could perhaps be reduced to 300 following a more detailed reconsideration of the UF_D value. Regardless of whether the value changes, a more detailed discussion would provide a better scientific justification of the value and this organ/system-specific Reference Concentration (osRfC).

- The EPA should consider adding a greater level of uncertainty for this osRfC(based on Glaser *et al.*, (1990) in Section 4.2.5 as an additional justification for selecting the osRfC based on human data from Gibb *et al.* (2000a) as the overall RfC.
- The EPA should define vague terms used to describe Cr(VI) uptake. Throughout the text (e.g., p 3-11), there is reference to the "rapid" cellular uptake of Cr(VI) versus "slow" cellular uptake; absent is any quantitative information on the magnitude of the difference between "rapid" and "slow."
- The EPA should clarify the discussion of pulmonary chromium particle deposition.
- The EPA should clarify the inconsistencies in the presentation of some studies on human lower respiratory effects of Cr(VI) as discussed above.
- The EPA should characterize the magnitude of the observed changes, specifically, whether they are considered to be mild vs. severe, etc., and whether they suggest a specific type of lung disease (e.g., obstructive, restrictive).
- The EPA should provide a more careful analysis in the bronchoalveolar lavage (BAL) response section of the draft IRIS assessment.
- The EPA should correct and more carefully present the description of the macrophage accumulation data from the Glaser *et al.* (1990) study.
- The EPA should clearly indicate whether the studies measured lung wet (total) weight or dry weight.
- The EPA needs to enhance the section that describes the regional particle deposition versus particle size (p. 3-39).

c. *Respiratory (noncancer nasal cavity):*

   i. *As noted in Appendix A (Systematic Review Protocol), a determination that **evidence demonstrates** Cr(VI) causes nasal lesions in humans was adopted from the 1998 draft IRIS assessment. A POD from Gibb et al. (2000a) was selected to calculate an osRfC of 1×10^{-5} mg/m^3 based on ulceration of the nasal septum. A composite uncertainty factor of 300 was used to account for intraspecies, subchronic-to-chronic, LOAEL-to-NOAEL, and database deficiency uncertainties. This osRfC was selected as the overall RfC. Please comment on whether the selection of the overall RfC is scientifically justified and clearly described.*

The panel agreed that the selection of the overall RfC is scientifically justified, although several issues were identified that would benefit from additional analysis and discussion. The charge question focuses on evaluation of the POD derived from Gibb *et al.* (2000a), not on the hazard identification (because the nasal hazard has been firmly established in prior documents). As outlined below, the panel believed the draft IRIS assessment should be strengthened by providing a more robust and effective description of the evidence supporting its scientific syntheses, interpretations, and conclusions.

The panel agreed that the POD for nasal effects derived from Gibb *et al.*, (2000a) should be reconsidered or at the very least more transparency is required. There is a lengthy discussion of why the results of Gibb *et al.* (2000a) are preferred over the other studies even though the other studies provide a lower $POD_{[HEC]}$ (Human Equivalent Concentration POD) or osRfC (Table 4-10

and starting on p. 4-46). This analysis may be incomplete due to the following elements. More clarity is required regarding the duration of employment among the several studies that were cited. The Gibb *et al.* (2000a) study indicated the time to appearance of symptoms was generally less than 1 year, whereas in the Lindberg and Hedenstierna (1983) study the median duration of exposure was 4.5 years with a maximal employment of 36 years. The Gibb study excluded women, but about 25% of the subjects in the Lindberg and Hedenstierna (1983) study were women, an advantage of using these data. More importantly, the Gibb *et al.* study focused on workers in a chromate production plant, whereas the other three study groups (Table 4-6) examined the chrome plating industry (see prior comment). *A priori*, one would expect there might be differences in the results from differing industries. Indeed, the $POD_{[HEC]}$ (Table 4-10) derived from the Gibb study for chromate production workers is consistently higher than that for workers in the chrome plating industry. Occam's Razor would suggest the simplest explanation is likely (i.e., there are different concentration-response relationships in chrome plating *versus* chromate production scenarios). If the aerosols within chromate production facilities are deemed to be more representative of environmental Cr(VI) aerosols, this should be explicitly stated as the basis for selecting Gibb et al. (2000a) rather than Lindberg and Hedenstierna (1983) for POD determination.

A cohort of 4 sets of studies was evaluated in the overall analysis (Table 4-6). An essential facet of these studies is that they examined workers in different industries that were exposed to different aerosols. Presumably, the electroplating workers were exposed to chromic acid aerosols, whereas the chromate production workers were exposed to a wide range of aerosols derived from roasting to water extraction processes. It appears that a single study regarding particle size was cited (Kuo *et al*., 1974 (p. 3-9)). This study relates to only one occupation – electroplating. This is inadequate to describe the particle sizes observed in other occupations. At the very least, this issue highlights the need for greater clarity in the IRIS assessment.

The conclusions in the draft IRIS assessment were based on the assertion that the response(s) to all Cr(VI) aerosols are similar, particularly with respect to concentration-response relationships. This conclusion is not supported by careful analysis of the data. The LOAEL in the Gibb *et al.* (2000a) study of 10.4 $\mu g/m^3$, which was based on exposures by chromate production workers (Table 4-10, p. 4-40), was many fold-higher than multiple other studies that examined workers in the chrome plating industry. This simple observation belies the conclusion that the response is the same for all aerosolized forms of Cr(VI). Perhaps, the draft IRIS assessment assumed that the aerosols in chromate production facilities are more representative of environmental Cr(VI) aerosols than the acidic aerosols in the electroplating industry. If so, transparency requires that this point be stated explicitly.

Furthermore, the EPA should indicate the particle sizes observed in these studies or explicitly indicate that the particle sizes are not known (p. 4-27 to 4-28). Also, stainless steel welding is not included in this group of studies, and one might anticipate that the particle sizes generated in welding operations might differ significantly from the other occupations due to the energy/heat involved in their generation. This deficiency in the database might be noted.

The EPA should reconsider their discussion of particle size and deposition. The paragraph on particle size (p. 4-48) is not scientifically supported (as are the comments in particle size on p. 4-

42). The statement about regional particle deposition patterns is too simplistic (see earlier comments). The draft IRIS assessment contains no precise information on the particle sizes in the epidemiological studies. The particle size in the human studies may or may not have been larger than that in the rodent studies. Also, the fact that dichromate salts are hygroscopic needs to be considered and is not in the draft IRIS assessment. Finally, and perhaps most importantly, the cited rodent studies did not include an assessment of nasal effects. Absence of data does not imply absence of response. Any statement about rodent vs. human sensitivity to nasal effects is without scientific foundation.

The panel noted a lack of transparency regarding the description of the results of Lindberg and Hedenstierna (1983). Table 4-10 states the LOAEL for nasal ulceration in the study is 2 $\mu g/m^3$. Yet, the subsequent text on p. 4-47 (line 24) states nasal ulceration was only seen in the highest peak exposure group (20-48 $\mu g/m^3$). A clarification to this point must be added to the assessment. If it is the latter, then why was a NOAEL not determined? Also, significant nasal atrophy was seen in the 2.5-11 $\mu g/m^3$ groups. Why is this not considered to be a toxic effect? The EPA should also provide more specific justification regarding nasal septum perforation as a Cr-specific effect. While the panel agreed that the strengths of using nasal septum perforation as an effect specific to Cr are well founded, the justification is too broad as written. Read literally, the draft IRIS assessment states that nasal mucosal atrophy and ulceration and perforation are specific to Cr (p. 4-27). This statement is not true as many inhaled toxicants cause nasal mucosal atrophy and ulceration.

The EPA should provide a better reference to support the characterization of the types of aerosols present in the workplace. In the description of the prior IRIS assessment (p. 4-47), the reference cited (Hayes *et al.*, 1979) did not include characterization of airborne dusts in the chromate industry. It only listed the raw materials used.

A UF_D of 3 was applied in deriving the candidate RfC based on the ulceration of the nasal septum observed in Gibb *et al.* (2000a) (Table 4-11, p. 4-44). Page 4-43 indicates that a UF_D value of 3 was applied because many of the inhalation studies were low confidence. The panel suggested that the EPA reconsider the UF_D value since other UFs are already being utilized. The EPA should recognize the interrelatedness of UFs and note the implications of mechanistic evidence (Section 3.2.1.3, p. 3-34 to 3-38) and other potentially relevant PK evidence regarding more sensitive remote effects. Also, the EPA should provide further discussion of the implications of PK considerations for the UF_D value. This discussion would serve to increase the scientific justification of the UF_D value, as well as the overall RfC value itself regardless of the final UF_D value. As an example of considering the value in the context of the other UFs (i.e, a UF_L of 10, UF_S of 3, and UF_D of 3) may be interpreted as tantamount to a judgment that given everything known about Cr(VI) PK and toxicity, there is an appreciable risk that a missing chronic study would identify a NOAEL POD or remote adverse effects as much as 100 times lower than the LOAEL POD used by EPA to derive the RfC.

The EPA should reconsider some of the UF values for noncancer respiratory nasal effects. With respect to UFs (p. 4-42 to 4-43), the rationale for using an UF of 3 for intraspecies uncertainties is clearly described. The interspecies UF of 1 is appropriate when using human data. The subchronic-to-chronic UF of 3 has a lengthy description. The comments related to particle size

are speculative, non-scientific, and non-transparent. The text provides sparse if any information on particle size within the various occupational settings and nothing about particle size in the ambient environment. If a comprehensive evaluation suggests limited effects of prolonged versus shorter exposure (similar to the lower respiratory tract), then an UF=3 would be appropriate. The LOAEL-to-NOAEL factor of 10 is appropriate.

The EPA needs to clarify the description of the UF for database uncertainty, as the current discussion is confusing (p. 4-43). Does the intra-species UF=10 incorporate issues relative to susceptible populations (e.g., women, under-age workers, non-working age persons, etc.)? If so, why are these concerns highlighted in the assessment? Also, the study of Lindberg and Hedenstierna included female workers. Is the primary concern the potential for non-portal of entry effects? Do any of the low confidence occupational studies indicate the possibility of non-portal of entry effects? Do pharmacokinetic considerations suggest that limited Cr(VI) is expected to penetrate to the bloodstream in the respiratory tract? A greater depth of discussion would enhance clarity and transparency about this issue.

While inhaled Cr(VI) is primarily distributed to the respiratory tract and can escape extracellular reduction to enter systemic circulation (Section 3.1.1), there is rapid uptake by red blood cells (RBCs) and reduction of Cr(VI) to trap Cr(III) within RBCs (Figure 3-1, p. 3-2). Moreover,for comparison to the inhalation LOAELs/BMDs available, any use of physiologically-based pharmacokinetic (PBPK) modeling to estimate the approximate inhalation exposures must achieve the same order of systemic distribution as the systemic effects caused through oral exposure (e.g., hematological, hepatic; Table 4-3).

Recommendations:

Tier 1:

- The EPA should provide an explicit description of what is known about the exposure characteristics, including particle size, in each study followed by a detailed consideration of this fundamentally important parameter.
- The EPA should provide a more careful examination of the apparent concentration-response relationships in differing Cr(VI) occupational exposure scenarios, with more transparency relative to the selection of the critical study (Gibb *et al.*, 2000a).
- The EPA should more thoroughly and transparently justify the selection of the Gibb *et al.* (2000a) study for POD determination.
- The EPA should provide greater clarity in evaluating the Lindberg and Hedenstierna (1983) study regarding the LOAEL/NOAEL of specific nasal responses.

Tier 2:

- The EPA should consider reexamining the UF_D value in more detail since other UFs are already being utilized. The EPA should also provide further discussion of the implications of PK considerations for the UF_D value.
- The EPA should provide a more robust justification regarding nasal septum perforation as a Cr-specific effect.

- The EPA should indicate the particle sizes observed in the cohort studies or explicitly indicate that the particle sizes are not known (p. 4-27 to 4-28).
- The EPA should reconsider some of the UF values for noncancer respiratory nasal effects and needs to clarify the description of the UF for database uncertainty.
- The EPA should expand their discussion of particle size and deposition.
- The EPA should provide a better reference to support the characterization of the types of aerosols present in the workplace.

d. Hepatic:

*i. The **evidence indicates** that Cr(VI) likely causes hepatic effects in humans given sufficient exposure conditions. This conclusion is primarily based on studies in animals that observed hepatic effects with increasing drinking water exposure levels. Increased clinical chemistry markers for liver dysfunction (ALT and AST), as well as increased chronic inflammation and fatty change were seen across animal studies. Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed that Cr(VI) clearly causes liver toxicity, given the elevation of liver enzymes seen following exposure and dose/response in liver toxicity in animal studies. Section 3.2.4 of the draft IRIS assessment focuses on hepatic effects of Cr(VI) (p. 3-161 to 3-186). The section is clearly divided into four subsections on human evidence, animal evidence, mechanistic effects, and integration of evidence.

The subsection on human evidence clearly explains why three of the four studies were rated as low confidence and one was rated as uninformative. The criteria used for these ratings are clearly and transparently presented. The draft IRIS assessment notes the existence of some inconsistency in the direction of results for total protein and albumin in two of the studies. Nonetheless, the clinical chemistry results allow for a conclusion that Cr(VI) exposure of workers is associated with statistically significant increases in serum chemistry markers of liver dysfunction.

The subsection on animal study evidence lists 18 studies whose results are categorized as high confidence, medium confidence, low confidence, or uninformative, based on the parameter being evaluated. A couple of the conclusions regarding liver histopathology and inflammation are presented with a fair bit of uncertainty and in a manner that contributes to a lack of confidence in the conclusion.

The subsection on mechanistic evidence summarizes data on oxidative stress, changes in gene expression, induction of apoptosis and necrosis, endoplasmic reticulum stress, and mitochondrial dysfunction. The draft IRIS assessment also notes that *in vitro* studies in human-derived cell lines support the applicability and biological plausibility of these results to humans. In the section on hazard identification (Section 3.3, p. 3-315), the draft IRIS assessment concludes the following: “The human evidence for Cr(VI)-induced liver effects is limited. Mechanistic evidence supports the hepatic effects observed in animals and humans and suggests a possible MOA of Cr(VI)-induced liver toxicity involving the production of free radicals and reactive

intermediates through intracellular Cr(VI) reduction resulting in oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis."

The panel agreed that these conclusions are scientifically justified and require only a few minor corrections for clarity. Despite a few concerns about clarity, the conclusions about the liver as a target organ for oral exposure to Cr(VI) are scientifically justified. Finally, the subsection on integration of evidence succinctly summarizes the human and animal evidence. The section ends with the conclusion: "Taken together, the serum enzyme and histopathology data from human, animal, and *in vitro* studies support biologically significant changes in the livers of rodents orally exposed to Cr(VI)."

ii. *A POD from NTP (2008), a 2-year drinking water bioassay in rodents, was selected to calculate an osRfD of 7 × 10^{-4} mg/kg-d based on chronic inflammation in female rats. A composite uncertainty factor of 100 was used to account for intraspecies, animal-to-human, and LOAEL-to-NOAEL uncertainties. Please comment on whether the selection is scientifically justified and clearly described.*

A 2-year drinking water bioassay in rodents is appropriate to calculate the osRfD of 7 x10^{-4} mg/kg based on chronic inflammation in female rats exposed to chromium. An uncertainty factor of 100 was applied to account for uncertainties and this approach is appropriate.

The section in the draft IRIS assessment on Oral RfD for effects other than cancer discusses hepatic toxicity, dose-response, and uncertainties on p. 4-6 to 4-7, 4-11, 4-18 (PDF p. 375-376, 381, 388). The design features of the various studies are summarized and the rationale for concluding that the NTP (2008) study is a high-confidence study is clearly explained. The draft IRIS assessment notes that dose-response modeling was performed on 5 parameters/responses involving liver effects:

1) Increased alanine transaminase (ALT) in male rats from NTP (2008) at 90-day and 12-month time points;
2) increased ALT in male and female rats from NTP (2007) and NTP (2008) at 90 days;
3) increased chronic liver inflammation in female rats from NTP (2008) at 2 years;
4) increased chronic liver inflammation in female mice from NTP (2008) at 2 years; and,
5) fatty liver change in female rats from NTP (2008) at 2 years.

The draft IRIS assessment explains, in section 4.1.2.3, which responses are amenable to BMD modeling and where a NOAEL and LOAEL could be identified. Section 4.1.3 (p. 4-12 to 4-18; PDF p. 382-388) then shows the calculations for the POD used to derive an osRfD value of 7 × 10^{-4} mg/kg-d based on chronic inflammation in female rats. The draft IRIS assessment clearly explains the rationale for this choice as it provides the lowest osRfD value with the lowest level of uncertainty. These explanations are scientifically justified, consistent with standard EPA policy and practices, and are clearly presented.

One panel member expressed some concerns about the scientific justification for the animal model chosen as the source of data for POD and osRfD determination. The specific comments are provided in the AppendixB.

Recommendations:

Tier 1:

- The utility of the ALT data for POD derivation is questionable and EPA should consider excluding them from the dose-response analyses.
- Although reasons for the difference in responses with route of exposure are addressed elsewhere, a brief summary and reference to where in the draft IRIS assessment this issue is discussed should be provided in the body of the document. For example, in discussing the clinical chemistry results in animals (p. 3-172, lines 14-20), it was noted that "significant increases in serum markers of liver damage were reported in several high and medium confidence oral exposure studies." However, the draft IRIS assessment then notes that "No effects on serum markers of liver damage were reported following inhalation exposures."

Tier 2:

- The EPA should provide more details and explanation for conclusions about the categorization of animal studies. The subsection on animal study evidence lists 18 studies whose results are categorized as high confidence, medium confidence, low confidence, or uninformative, based on the parameter being evaluated. A couple of the conclusions regarding liver histopathology and inflammation are presented with a fair bit of uncertainty and in a manner that contributes to a lack of confidence in the conclusion.
- The EPA should provide additional discussion about the validity of the conclusion that female rodents are more sensitive than male rodents. On p. 3-169, lines 11-13: The draft IRIS assessment states: "In general, female rodents appear to be more sensitive to Cr(VI) induced histological changes (e.g., hepatic inflammation and fatty changes; NTP (2008)). However, few studies are available in the database that evaluated both males and females…".
- The EPA should provide additional discussion about the absence of increases in chronic inflammation and histiocytic infiltration in male mice. The draft IRIS assessment states (p. 3-169, lines 20-23): "For mice, which generally appeared to be less sensitive than rats to hepatic effects with Cr(VI) exposure, statistically significant increases in chronic inflammation and histiocytic infiltration were seen in female, but not male mice (NTP, 2008)."

e. Developmental:

*i. The **evidence indicates** that Cr(VI) likely causes developmental effects in humans given sufficient exposure conditions. This conclusion is primarily based on the observation of decreased offspring growth across most animal studies, as evidenced by decreased fetal or postnatal body weights and decreased skeletal ossification. Other outcomes in animal studies are more uncertain because they were inconsistent among high and medium*

confidence studies or were evaluated only in low confidence studies. Likewise, the available human data were of low confidence and difficult to interpret. Please comment on whether this conclusion is scientifically justified and clearly described.

While the body of evidence for the developmental effects of Cr(VI) comes mainly from animal studies, the number of human studies is probably larger than the ones summarized in Table 3-45. Additional studies that can be included are listed under "Recommendations: Tier 3" below.
It is likely that Cr(VI) causes developmental toxicity based on decreased offspring growth across most animal studies, decreased fetal and body weights and decreased skeletal ossification. Because of decreased offspring growth (e.g., fetal and postnatal body weights) across most animal studies, Cr(VI) was deemed to affect developmental progression. Human data were of low confidence and difficult to interpret. The panel agreed that the overall conclusion on the developmental effects from Cr(VI) exposure is scientifically justified and clearly described.
The use of questionnaires to assess past and current welding exposure in the Hjollund *et al.* studies (2005, 2000, 1995) and JP *et al.* (1992) does not necessarily mean the exposure assessment is unreliable. Exposure misclassification may be unavoidable, but the general quality of exposure assessment in occupational studies using questionnaires may still be useful. Thus, the "low" confidence designation for these studies may be unnecessary.

Considering the human studies reviewed and the newer studies listed under Tier 3 recommendations below, the overall interpretation may still be inconclusive with spontaneous abortion, preterm birth, reduced fetal growth, and infant death. While the conclusion in the draft IRIS assessment may not change, these studies can elevate the importance of the human studies and support the uncertainty in the determination of Cr(VI) from Cr(III) exposure and differentiation of specific effects attributable to chromium in the context of metal mixtures. It is not prudent to dismiss some of the positive findings although clearly the conclusion is imprecise and with considerable uncertainty. On the technical side, the composite outcome of preterm birth and fetal growth restriction may make the outcome less comparable with other studies. Pregnancy loss is mostly defined as a loss within 20 weeks of gestation in the U.S. studies, while international studies may consider a loss at less than 28 weeks of gestation. Preterm birth is one of the major reasons of low birth weight, but the preferred outcomes would be separating preterm birth and small-for-gestational-age, rather than the use of low birth weight.

Regarding the evidence from animal studies, it is interesting to note that 2 high and 1 medium confidence studies reported no effect on pre- and post-implantation loss while 10 low confidence studies reported loss. The reason for this discrepancy is unclear. Rather than dismissing the low confidence studies, these studies should be scrutinized to understand the difference in the observed effects. Reduced postnatal growth was more consistently found in animal studies, including 1 out of 2 high confidence and 8 of 9 low confidence studies. The reason that the high confidence study by Zheng *et al.,* (2018) and the sole medium confidence study by De Flora *et al.,* (2006) did not find effects on F1 postnatal growth, was not clear. Despite this outcome, the observed effect on F1 postnatal growth appears to be convincing. Reduced skeletal ossification was observed in all low confidence studies, in line with the findings of reduced body weight. Even without medium or high confidence studies, the consistency of the results suggests the potential of an effect on the bone formation processes. The occurrence of placenta histopathology was suggested in studies, but quantitative findings were not available. The

placenta weight was not consistently found to be affected by Cr(VI) exposure. It is reasonable to assume that the results regarding placenta weight and function are not conclusive.

The draft IRIS assessment states (p. 3-283) that "Four studies were found to be uninformative due to critical deficiencies in one or more domains (Xia *et al.*, 2016; Quansah and Jaakkola, 2009; Ren *et al.*, 2003; Chen *et al.*, 1997) and were not considered further." In this section, it would be informative to explain in more detail why these studies were found to be uninformative, as this information was included in other parts of the draft IRIS assessment (e.g., completed for epidemiological studies and animal studies). Without this information, it is difficult to fully understand the rationale for the decisions made.

Comments from one panel member raised questions about the value of using ecological studies and the appropriateness of interpretations from epidemiological studies in the area of developmental effects. These comments are presented in the Appendix B.

In summary, the panel agreed that the overall conclusion on the developmental effects from Cr(VI) exposure is scientifically justified and clearly described. Incorporating newer human studies may add clarity to the findings related to birth weight and preterm birth in humans, but inconsistency may still persist given the discrepancies between these study findings.

ii. *A POD from NTP (1997), a continuous breeding study in BALBC mice, was used to derive an osRfD of 0.07 mg/kg-d based on decreased F1 offspring postnatal growth. A composite uncertainty factor of 10 was used to account for intraspecies and animal-to-human uncertainties. It should be noted that the decreased F1 offspring growth effect was observed at maternal dose of 24.4 mg/kg-d, which is a relatively high dose associated with overt toxicity in other studies. Both indirect (maternal or paternal) and direct routes of exposure to the developing organism were considered during hazard assessment. It is frequently difficult to determine whether effects on the fetus are in response to or separate from maternal toxicity in studies that report both, and so the fetal endpoints were considered in conjunction with the maternal endpoints described in the "Female reproductive effects" section. Developmental effects at doses that cause minimal maternal toxicity are still considered to represent developmental toxicity and should not be discounted as maternal toxicity U.S. EPA (1991). However, because this effect only occurred in high dose groups where other toxicological effects (as indicated by the lower points of departure for other toxicities) may be occurring, this osRfD was assigned low confidence. Please comment on whether the selection is scientifically justified and clearly described.*

The selection of organ-specific RfD (osRfD) for development toxicity (0.07 mg/kg-d) is scientifically justified and clearly described. The NOAEL was used to derive POD_{HED} (0.7 mg/kg-d) instead of the LOAEL. The uncertainty factor of 10 (interspecies 3 and intraspecies 3) was used, as the exposure was in the sensitive window. Thus, the UF was set as 10.

Recommendations:

Tier 2:

- The EPA should reevaluate the "low confidence" designation for the Hjollund *et al.* (2005, 2000, 1995) and JP *et al.* (1992) studies.
- In this section, the EPA should explain in more detail why these studies by Xia *et al.*, 2016; Quansah and Jaakkola (2009); Ren *et al*, (2003); and Chen *et al.* (1997) were found to be uninformative, as was done in other parts of the draft IRIS assessment for epidemiological studies and especially animal studies.
- The EPA should better explain and justify the inclusion of two studies (i.e., p. 3-284, Remy *et al*., 2017; Eizaguirre-García *et al*., 2000) using geographically-based measures of exposure.
- The EPA should interpret the epidemiological evidence as unsupportive of an association with developmental outcomes due to significant study limitations and inconsistent findings, rather than calling the evidence "slight" and "uncertain."
- The EPA should more carefully interpret data on fetal and postnatal growth (Section 3.2.9) due to discrepancies between results of various studies. The conclusion about the effect of chromium on developmental outcomes based on these specific responses may need to be revised.

Tier 3:

- The low confidence animal studies should be more thoroughly scrutinized to understand the difference in the observed effects between them and the two high and one medium confidence animal studies that had differing conclusions regarding pre- and post-implantation loss.
- The EPA should incorporate the following newer human studies in this assessment. Doing so may add clarity to the birth weight and preterm birth findings in humans. Yet, inconsistency may still persist given the discrepancy in these study findings.

    - Pregnancy Outcome in Women Exposed to Metal Fume in Welding: A Canadian Cohort Study. Available at: https://pubmed.ncbi.nlm.nih.gov/35488367/
    - Maternal exposure to metal mixtures during early pregnancy and fetal growth in the Jiangsu Birth Cohort, China. Available at: https://pubmed.ncbi.nlm.nih.gov/36096164/
    - Prenatal metal(loid) mixtures and birth weight for gestational age: A pooled analysis of three cohorts participating in the ECHO program. Available at: https://pubmed.ncbi.nlm.nih.gov/35081493/
    - Exposure to atmospheric metals using moss bioindicators and neonatal health outcomes in Portland, Oregon. Available at: https://pubmed.ncbi.nlm.nih.gov/34030082/
    - Associations of prenatal exposure to multiple metals with testicular volume and anogenital distance in infant boys: A longitudinal cohort study. https://pubmed.ncbi.nlm.nih.gov/32653800/
    - Birth outcomes associated with maternal exposure to metals from informal electronic waste recycling in Guiyu, China. Available at: https://pubmed.ncbi.nlm.nih.gov/32078870/

- Placental metal concentrations and birth outcomes: The Environment and Childhood (INMA) project. Available at: https://pubmed.ncbi.nlm.nih.gov/30638867/

f. Hematological:

*i. **Evidence suggests** that Cr(VI) may cause hematological effects in humans given sufficient exposure conditions. This conclusion is based primarily on moderate animal evidence from high and medium confidence subchronic and chronic studies in rats and mice reporting consistent, dose-related, and coherent findings at 22-90 day exposures. However, the magnitude of the collective effects decreased by 12 months, with many findings returning to normal or near normal levels. Organ/system-specific reference doses were derived based on short-term hematological effects because factors demonstrated a credible concern for greater toxicity in a susceptible population and life stage (individuals with iron-deficient anemia, and pregnant women who are susceptible to developing iron-deficient anemia). Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed that with sufficient exposure conditions, Cr(VI) causes hematological toxicity, which was enhanced with iron-deficient anemia and during pregnancy. This conclusion was appropriate.

The draft IRIS assessment states that "overall, the currently available evidence suggests that Cr(VI) exposure may cause hematologic effects in humans" (p. 3-197, lines 31-32). Table 3-31 (p. 3-199 to 3-201) is the evidence profile table for hematological effects. This table generally contains factors that both increase certainty (e.g., consistent findings of decreased Hgb, MCH, MCHC, MCV, and increased RBC) and decrease certainty (e.g., lack of duration-dependence) along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary conclusion. While the human evidence for hematological effects is indeterminate, the laboratory animal evidence is moderate. The draft IRIS assessment (Section 3.2.5) also contains information relevant to and supporting the weight-of-evidence for hematological effects.

ii. A POD from NTP (2008), a 2-year drinking water bioassay in rodents, was selected to calculate an osRfD of 0.01 mg/kg-d based on decreased hemoglobin in male rats reported at 22 days. A composite uncertainty factor of 10 was used to account for intraspecies and animal-to-human uncertainties. A subchronic-to-chronic uncertainty factor was not applied, because this effect was observed to ameliorate with chronic exposure. Please comment on whether the selection is scientifically justified and clearly described.

The panel considered an osRfD of 0.01 mg/kg calculated based on a 22-day male rat study and the use of an uncertainty factor of 10 are appropriate.

One panel member noted that only rat data were considered for derivation of an osRfD for hematological effects (Table 4-3, p. 4-13), although there are multiple high confidence studies in

both mice and rats (Table 3-30, p. 3-191 to 3-192). The concern was expressed that the choice of species from which the POD and RfD were derived may not be fully explained or appropriate without additional analysis.

g. Immune:

> ***Evidence suggests*** *that Cr(VI) may modulate the immune system in humans, through both stimulatory and suppressive actions, given sufficient exposure conditions. 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. No reference values were derived for this system. Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed that both animal and human studies indicate that Cr(VI) has suppressing effects on the immune system. However, some of the studies available in the literature are of "low confidence" with findings that show differing effects depending on the study. The panel agreed that the conclusions are sufficient and justified based on the available studies.

h. Male reproductive:

> ***Evidence suggests*** *that Cr(VI) may cause male reproductive toxicity in humans given sufficient exposure conditions. This conclusion is primarily based on coherent evidence of effects across human and animal studies. Decreased testosterone and decreased sperm quantity and quality were observed in both human and animal studies; however, interpretation of this evidence was limited because most studies that observed these effects were considered low confidence and there was inconsistency with higher confidence studies. No reference values were derived for this system. Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed that in general, the available data on male reproductive effects are clearly and appropriately synthesized to describe the strengths and limitations. The draft IRIS assessment on page 3-253, line 36 states that "overall, the evidence suggests that Cr(VI) may cause male reproductive toxicity in humans." Table 3-42 (p. 3-256 to 3-262) is the evidence profile table for male reproductive effects, which among other information generally contains factors that both increase certainty (e.g., organ weight changes coherent with decreased testosterone within low confidence studies) and decrease certainty (e.g., unexplained inconsistency for organ weight across high confidence studies) along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary conclusion. The human evidence for male reproductive effects is slight, as is the laboratory animal evidence. The draft IRIS assessment (Section 3.2.7) also contains information relevant to and supporting the weight-of-evidence for male reproductive effects.

Taken together, the weight of the available scientific information presented reasonably supports that with sufficiently high exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions" as stated in Table 3-42), Cr(VI) exposure may cause male reproductive toxicity in the general human population, including potentially susceptible subpopulations. The bases for this weight-of-evidence decision are clearly described in the text (i.e., *Integration of Evidence* on pp. 3-253 and 3-255) and Table 3-42 of the draft IRIS assessment.

Recommendations:

Tier 2:

- EPA needs to clarify how they developed the suggestive conclusion given sufficient exposure, because EPA also says the "evidence of an association between Cr(VI) exposure and male reproductive effects in humans is slight." Similarly, that evidence was slight in animal studies and mechanistic studies. While the review panel agrees that the evidence is suggestive, the addition of the statement "may cause male reproductive toxicity in humans given sufficient exposure conditions" in humans can be confusing because it implies causality. "May potentially" is a better way to phrase this statement. This comment pertains to other places in the draft IRIS document using the same terminology.

i. Female reproductive:

***Evidence is inadequate** to assess whether Cr(VI) may cause female reproductive effects in humans. Although an association with female reproductive toxicity was demonstrated in a single low confidence epidemiology study and a series of low confidence animal toxicology studies, effects were not observed in medium or high confidence studies aside from a moderate decrease in maternal body weight. No reference values were derived for this system. Please comment on whether this conclusion is scientifically justified and clearly described.*

The panel agreed with EPA's conclusion that the evidence is inadequate to assess whether Cr(VI) may cause female reproductive effects in humans. The available data on female reproductive effects are clearly and appropriately synthesized to describe the strengths and limitations. Table 3-44 (pp. 3-277 to 3-282) of the draft IRIS assessment presents the evidence profile table for female reproductive effects, which among other information generally contains factors that both increase certainty (e.g., consistency in decreased maternal body weight across studies) and decrease certainty (e.g., low confidence studies did not adjust for gravid uterine weight) along with evidence stream (i.e., human, animal, mechanistic) judgments/rationales and a summary conclusion. The human evidence for female reproductive effects is indeterminate, as is the laboratory animal evidence. Further, the draft IRIS assessment (Section 3.2.8) also contains information relevant to and supporting the weight-of-evidence for female reproductive effects. Taken together, the weight of the available scientific information presented reasonably supports the conclusion that the evidence is inadequate to assess whether Cr(VI) causes female reproductive toxicity in humans (as stated in Table 3-44). The bases for this weight-of-evidence

decision are clearly described in the text (i.e., *Integration of Evidence* on pp. 3-275 and 3-276) and Table 3-44 of the draft IRIS assessment.

Charge Question #4 - Benchmark Dose Modeling

EPA used benchmark dose (BMD) modeling to identify points-of-departure (PODs) for the following Cr(VI)-induced health effects observed in rodents: respiratory, gastrointestinal (cancer and noncancer), and hepatic. Are the modeling approaches used, selection and justification of benchmark response levels, and the selected models used to identify each POD for toxicity value derivation scientifically justified and clearly described?

The panel agreed that, in general, the modeling approaches, model selection process, and benchmark response (BMR) levels used to identify PODs for toxicity value derivation for both non-cancer and cancer effects follow EPA guidance and are scientifically justified. They also agreed with EPA's rationale for concluding that a NOAEL or LOAEL should be used as the POD because BMD modeling is not supportable for some endpoints.

EPA's approach for converting the oral dose in rodent studies to the Human Equivalent Dose (HED) for non-cancer and cancer effects in the small intestine and non-cancer systemic effects from oral exposure involved determining the internal dose in rodents. First, EPA determined the dose that escaped reduction in the stomach with a human PK model and then adjusted by $BW^{3/4}$ (i.e., $[BW_A/BW_H]^{1/4}$) to account for interspecies differences in the volume of the small intestine. The relevant human administered dose (i.e., HED) was then back-calculated from the human internal dose with a human PBPK model for human reduction of Cr(VI) in the stomach. For reasons explained in the draft IRIS assessment, the HED was based on the lower 1% value of the administered dose from the human PBPK model for non-cancer effects and the 50% value for carcinogenic effects. This rather complex approach for determining the HED was developed specifically for oral exposure to Cr(VI), and it was not necessarily intuitive as to why both the PK model and the $BW^{3/4}$ adjustments were made.

Additionally, as will be discussed in the response to Charge Question 6, the majority (13 of the 14) of the panel members recommended that the EPA further consider using toxicokinetic principles (specifically, dose-dependency in the fraction of chromium dose accumulated) in low dose extrapolation for the oral route of exposure and also stated that target tissue absorbed dose should be considered as a dose metric for modeling gastrointestinal tumors. One member of the panel noted that the rationale for linear low-dose extrapolation for the oral route as presented in the draft IRIS assessment was sufficient.

Also relevant to Charge Question 6, the panel recommended that the EPA consider models that are sublinear over the lower part of the dose-response, as well as the multistage degree 1 model selected by EPA for dose-response modeling of the mouse small intestinal tumor data if/when oral dose is the dose metric. The panel agreed that PODs (female – BMDL; male – LOAEL) for diffuse epithelial hyperplasia in the mouse small intestine were developed using appropriate

approaches. The panel noted that these PODs are already presented in the draft IRIS assessment and agreed that selection of the POD for this effect in females was the correct basis for derivation of the final RfD. Furthermore, it was noted that the draft IRIS assessment already includes an evaluation of an alternate threshold MOA based on this RfD (Section D.3.3 in the draft IRIS Supplemental Information document).

Recommendations:

Tier 1:

- The explanation for the approach in *Section 4.2.1.1 PBPK Modeling and Animal-to-Human Extrapolation* in the draft IRIS assessment should be expanded so that the rationale for using both adjustments to determine the internal human dose is more evident. A clear explanation was provided on p. C-17 in Section C.1.5.1 of Appendix C in the Supplementary Information, and this explanation could be used as the starting point for revision within the main draft IRIS assessment.
- Although Charge Question 6 addresses only cancer effects, the panel agreed that the recommendation noted above for the EPA to further consider using toxicokinetic principles (specifically, dose-dependency in the fraction of chromium dose accumulated) also applies to the dose-response modeling for non-cancer effects that is considered here in Charge Question 4.

Charge Question #5 - Uncertainty Factors (UFs)

EPA applied a series of five UFs to the POD developed for each noncancer related endpoint/study, specifically addressing the following areas of uncertainty: intraspecies uncertainty (UF_H) to account for variation in susceptibility across the human population, and the possibility that the available data may not be representative of individuals who are most susceptible to the effect; interspecies uncertainty (UF_A) to account for animal-to-human extrapolation, and consisting of equal parts representing pharmacokinetic and pharmacodynamic differences; subchronic-to-chronic uncertainty (UF_S) to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure, and to consider whether lifetime exposure would have effects at lower levels (e.g., for studies other than subchronic studies); LOAEL-to-NOAEL uncertainty (UF_L) to infer an exposure level where effects are not expected when a POD is based on a lowest-observed-adverse-effect level (LOAEL); and database uncertainty (UF_D) to account for database deficiencies if an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or life stage.

a. Has uncertainty been adequately accounted for in the derivation of the reference values? Please describe and provide recommendations, if needed.

The panel agreed that the draft IRIS assessment generally provides an adequate description of the choices made and the uncertainty factor (UF) types (i.e., UF_H - to account for variation in susceptibility across the human population; UF_A -to account for animal-to-human extrapolation; UF_S - subchronic-to-chronic uncertainty; UF_L - LOAEL-to-NOAEL uncertainty; and UF_D - to account for database deficiencies) for each toxicity value and study type. The panel has the following specific recommendations.

Recommendation:

Tier 2:

- The panel recommended that the IRIS assessment should mention on p. 4-14, lines 22-30 that the EPA (2011) guidance document, *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose*, states that an interspecies UF of 3 instead of the default UF_A of 10 should be applied when body weight$^{3/4}$ scaling is used.

b. To inform uncertainty in intraspecies variability, UF_H, the assessment evaluates and considers the available evidence on potential susceptibility to Cr(VI) within different populations or lifestages, including any potential human health impacts from early life exposure. Monte-Carlo analysis using pharmacokinetic modeling was applied to account for pharmacokinetic variability in the average/general adult population following oral exposure. As a result, for effects via the oral route, the UF_H was lowered from 10, and 3 was retained for pharmacodynamic variability. However, there may be residual pharmacokinetic variabilities for susceptible populations outside the capabilities of the standard adult-based model. These cannot be quantified and are discussed qualitatively in the assessment. Is the rationale for a UF_H of 3 scientifically justified and clearly described?

Even though there is potentially residual pharmacokinetic variability not accounted for by the PBPK model, the panel agreed that a UF_H of 3 to account for pharmacodynamic variability in the development of the RfD is appropriate and sufficiently protective. This relationship is particularly true because the POD is based on the lower 1% value from the Monte Carlo simulations. This selection is a conservative choice for this admittedly minimally adverse effect (Section 4.1.2.2, p. 4-10).

Recommendation:

Tier 2:

- If the overall intraspecies variability, including the more susceptible populations, is still accounted for by a UF_H of 3, this needs to be more clearly explained in the text.

c. A database uncertainty factor, UF_D, of 3 was applied to inhalation respiratory effects (both human nasal and animal lower respiratory). A value of less than 10 was applied because

respiratory tract effects of inhaled Cr(VI) are considered portal-of-entry effects, and are therefore likely to be amongst the most sensitive based on current understanding of pharmacokinetics and mechanisms following inhalation. A value of UF_D =3 (as opposed to UF_D =1) was applied because many of the inhalation studies were low-confidence (particularly for noncancer effects outside the portals of entry) and limited in scope (working-age and mostly male humans, and only male rodents). Due to pharmacokinetic differences from oral exposure (Cr(VI) is detoxified in the gut and liver on first-pass), the stronger oral database (UF_D=1 for all effects following oral exposures) could not be used to inform the UF_D for inhalation effects. Is the rationale scientifically justified and clearly described?

The panel concluded that the UF_D value of 3 should be reevaluated more comprehensively in the context of the other UFs already being used, as well as the implications of mechanistic evidence (Section 3.2.1.3, pp. 3-34 to 3-38) and other relevant PK evidence with regard to the potential for more sensitive remote effects. There is the real possibility that a missing chronic study might identify a NOAEL for POD or remote adverse effects as much as 10 times or more lower than the LOAEL POD used by EPA to derive the RfC unless it can be strongly argued from PK/mechanistic evidence that this is unlikely.

Relevant PK factors include (but are not limited to) considerations such as, while inhaled Cr(VI) is primarily distributed to the respiratory tract and can escape extracellular reduction to enter systemic circulation (Section 3.1.1), there is rapid uptake by red blood cells (RBCs) and reduction of Cr(VI) to trap Cr(III) within RBCs (Figure 3-1, p. 3-2). The ability of PBPK modeling to estimate the approximate inhalation exposures required to achieve the same order of systemic distribution as systemic effects resulting from oral exposure (e.g., hematological, hepatic; Table 4-3) should also be compared with the inhalation LOAELs/BMDs available. A discussion of the implications of these types of considerations for the UF_D value would serve to increase the scientific justification of the UF_D value and the overall RfC value. Further, the relevant database of human studies evaluating similar endpoints and a range of exposure types with similar lesions is actually quite extensive given the type of exposures. For example, the study by Gibb *et al.* was a relatively large occupational cohort study with a sample size of 2,307. Furthermore, exposure resulted in what was clearly a portal-of-entry type of pathology. Hence, the database is strong, especially for human data.

<u>Recommendations</u>:

<u>Tier 2:</u>

- The panel suggests that in light of the considerations highlighted above, a UF_D value of 1 is more appropriate for human inhalation PODs.

d. A subchronic to chronic uncertainty factor, UF_S, of 3 was applied to human nasal effects. While data were not from chronic lifetime exposures, the nasal effects were observed to have a short onset time. This may indicate that nasal effects occur following short-term occupational exposures to high concentrations of Cr(VI), when significant impaction of

large particulates or mists containing Cr(VI) occurs along the nasal passages. Based on the available evidence, it is considered less likely that exposure to Cr(VI) outside of occupational settings (where particulates are larger) would induce nasal perforations/ulcerations at much lower concentrations and smaller particle sizes. As a result, a factor of UF_S<10 was applied. Because it is possible that prolonged exposures to high concentrations may increase the severity of existing nasal lesions after they occur, a value of UF_S=3 (as opposed to UF_S=1) was applied. Is the rationale scientifically justified and clearly described?

EPA has provided a rationale for the UF_S of 3, although the justification could perhaps be clarified and/or strengthened. The panel noted that the first reason cited above is the strongest. The panel agreed that it is less likely exposure to Cr(VI) outside of occupational settings (where particulates are larger) would cause nasal perforations/ulcerations at much lower concentrations and smaller particle sizes. The second reason cited (for not using a UF_S value of 1) seems to assume that Cr(VI)-induced nasal lesions will occur in the environmentally-exposed population but can be made less severe by a UF of 3 (i.e., the intent of using a value of 3 seems to be to reduce the severity of nasal lesions induced by Cr(VI) rather than protecting against them in the first place).

EPA should consider adding further justification for not using a value of 1. As a starting point, perhaps EPA could consider something along the lines of, "However, a value of 3 is retained as there is residual uncertainty regarding the possibility that somewhat lower long-term environmental Cr(VI) exposure could induce adverse nasal effects or exacerbate preexisting nasal conditions in susceptible subpopulations (e.g., those prone to epistaxis, particularly anterior nosebleeds from the Kiesselbach's plexus (also known as Little's area) on the anterior nasal septum)."

Recommendation:

Tier 2:

- The EPA should consider adding further justification for not using a UF_S value of 1 as described above.

Charge Question #6 - Carcinogenicity Hazard Identification and Toxicity Value Derivation

For each cancer-related health effect and decision outlined below, please comment on whether the available data have been clearly and appropriately synthesized to describe the strengths and limitations and to support the conclusions presented. For each, please also comment on whether the weight-of-evidence decisions for hazard identification have been clearly described and scientifically justified. In addition, please separately comment on whether the dose-response decisions are transparent and scientifically justified, including study selection for dose-response

analyses; point of departure (POD) estimates, including modeling choices and assumptions, and dosimetric adjustments; derivation of candidate values; and confidence in the calculated values.

a. *EPA concluded that a mutagenic MOA for Cr(VI) carcinogenicity is "sufficiently supported in (laboratory) animals" and "relevant to humans." The determination applies to both oral and inhalation exposures. For inhalation, there was consistent evidence from humans exposed occupationally. For the oral route of exposure, the small evidence base of low confidence animal mutagenicity studies of drinking water exposures was supported by strong evidence of mutagenicity of Cr(VI) in test systems using more direct exposure methods (e.g., i.p. injection, in vitro) and a biologically plausible pharmacokinetic mechanism for Cr(VI) distributing to tumor target tissues and being taken up and reduced intracellularly to induce toxic and genotoxic effects.*

A large majority of the panel (12 of 14 members) agreed that the evidence for Cr(VI) causing cancer through a mutagenic mode of action (MOA) was sufficiently supported in experimental systems and was relevant to humans. The panel noted that while the rationale supporting the conclusion had been clearly stated, there are many additional genotoxicity and mutagenicity studies, particularly from *in vitro* test systems and in model organisms (other than laboratory rodents), that would substantially strengthen the case for the mutagenic MOA. The panel, therefore, recommends that these studies be added to the draft IRIS assessment.

The panel noted that MOA-related sections were clearly organized and that the strengths of the *in vivo* studies, along with the reasons for their classification as low, moderate, or high confidence, in most cases, were clearly explained. The MOA sections varied somewhat in their degree of emphasis on the importance of different mechanisms of Cr(VI) carcinogenicity, which is partially reflective of divergent opinions in the field and a more general familiarity and understanding of other mechanisms of genotoxicity (e.g., oxidative stress) than Cr-specific DNA damage. More emphasis on Cr(VI)-specific genotoxic effects such as Cr-DNA adducts and crosslinks would strengthen the case for the mutagenic MOA. Additional details and examples of areas where the MOA sections of the draft IRIS assessment could be strengthened can be found in Appendix B, for Charge Questions - 6a. Some limitations are presented in the draft IRIS assessment with additional limitations raised in the public comments. Also as indicated in the draft IRIS assessment, multiple MOAs for carcinogenicity could be occurring.

Overall, the panel agreed that the weight-of-evidence decisions for hazard identification (i.e., that Cr(VI) can cause cancer through both the inhalation and oral routes) have been clearly described and scientifically justified. As indicated in the draft IRIS assessment and in the public comments, less mechanistic evidence is available for the oral route of exposure, and recent reports of negative *in vivo* genotoxicity studies in the mouse intestine are not consistent with the substantial number of other positive genotoxicity studies.

However, the panel largely agreed with the EPA that these recent negative genotoxicity studies had significant deficiencies that reduced confidence in the studies and their conclusions, and that EPA's categorization of these studies as "low confidence" is appropriate. Several of the panel members expressed different views on the mode of action and other responses related to Charge Question 6. Their minority opinions can be found in Appendix B.

Recommendations:

Tier 1:

- The EPA should include additional genotoxicity and mutagenicity studies, particularly from *in vitro* test systems and in model organisms (other than laboratory rodents), that would substantially strengthen the case for the mutagenic MOA. More emphasis on Cr(VI)-specific genotoxic effects, such as Cr-DNA adducts and crosslinks, would also strengthen the case for the mutagenic MOA.

b. *Because tumors in rodents and humans were observed in (or proximal to) portals of entry where cellular uptake of Cr(VI) may occur prior to detoxification to Cr(III), and because a mutagenic MOA for Cr(VI) carcinogenicity is "sufficiently supported in (laboratory) animals" and "relevant to humans," EPA applied a low-dose linearity approach for both the oral and inhalation routes of exposure. In the absence of chemical-specific data to evaluate differences in age-specific susceptibility, increased early-life susceptibility to Cr(VI) is assumed and EPA applied age-dependent adjustment factors (ADAFs) in accordance with the Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens U.S. EPA (2005).*

The panel agreed that the dose-response decisions were transparent and recognized that the choice of linear extrapolation to estimate risk in the low dose region for both inhalation and oral exposures was consistent with EPA guidance. However, the panel noted, particularly for oral exposures, that there were multiple toxicokinetic factors that could significantly affect the shape of the dose response curve in the low to ultra-low dose region; and therefore, additional evaluation of these factors is warranted. Additional discussion of these points can be found in Appendix B. Based on these considerations, the majority of the panel recommended that the EPA further consider using toxicokinetic factors (specifically, dose-dependency in chromium accumulation) in low dose extrapolation for the oral route of exposure. One panel member supported the use of linear low-dose extrapolation for the oral route as presented in the draft IRIS assessment.

Recommendations:

Tier1:

- The majority of the panel recommended that the EPA further consider using toxicokinetic factors (specifically, dose-dependency in chromium accumulation) in low dose extrapolation for the oral route of exposure.

Tier 2:

- The panel observed that age-dependent exposure to different media such as soil and water should be incorporated into the application of age-dependent adjustment factors (ADAFs) when health-based criteria for such specific media are developed.

c. *EPA concluded that for cancer via the oral route of exposure, Cr(VI) is likely to be carcinogenic to the human GI tract. This conclusion is primarily based on robust evidence of cancer from a high confidence 2-year cancer bioassay conducted by NTP, which showed a statistically significant increase in oral cavity tumors in male and female F344/N rats and small intestine neoplasms in male and female B6C3F1 mice NTP (2008).*

All panel members agreed that the carcinogenicity data for the oral route of exposure support the conclusion that Cr(VI) is likely to be carcinogenic to the human GI tract according to the criteria in the EPA (2005a) Guidelines for Cancer Risk Assessment. In general, the panel maintained that the data had been clearly and appropriately synthesized to describe the strengths and limitations, and that the rationale and weight of evidence for the EPA's conclusion are transparent and scientifically justified. The panel agreed that the 2-year cancer bioassay conducted by NTP is the key available study. While there was agreement on major points, some issues were raised by one panel member which can be found in Appendix B- for Charge Question 6c.

d. *A POD from NTP (2008), a 2-year drinking water bioassay in rodents, was selected to calculate a total lifetime OSF for Cr(VI) of 0.5 (per mg/kg-d) based on increased incidence of adenomas and carcinomas in the small intestine of male and female mice. This value includes application of ADAFs.*

The majority of the panel agreed with the selection of the study, endpoint, approach, and POD used by the EPA to calculate low dose risks of Cr(VI); however, if through additional modeling, the EPA concludes that another approach is more scientifically supportable, then that approach should be used. The panel noted that the available data for the POD and the OSF have been clearly and appropriately synthesized to describe the strengths and limitations and do support the conclusions presented. However, the panel recommended that EPA should provide additional information on their rationale for choosing the mouse dataset (i.e., adenomas and carcinomas in the small intestine of male and female mice) as most representative of the overall dose-response in humans.

Most of the panel maintained that the weight-of-evidence decisions for hazard identification underlying this choice have been clearly described and scientifically justified. As discussed in the response to Charge Question #6b above, the rationale for applying ADAFs was considered to be transparent and scientifically justified, although some questions were raised. Additionally, the panel recommended that the discussion about the application of ADAFs to adjust the slope factor of 0.3 (per mg/kg-d) to 0.5 (per mg/kg-d) should be clarified to state that the slope factor of 0.3 (per mg/kg-day) applies to risks from less-than-lifetime exposures that occur only in adulthood, such as occupational exposures.

Additionally, the panel recommended that the draft IRIS assessment be revised to clearly indicate that the modeling of the tumors in males, as well as the modeling of the tumors in females, resulted in the same slope factor. The wording in the draft IRIS assessment (p. 4-54, lines 1-2) states, "The OSF for Cr(VI) was derived from small intestine tumors in male and female mice using PBPK modeling, 0.3 $(mg/kg\text{-}d)^{-1}$," may be misinterpreted to mean that the

slope factor is based on modeling of the combined tumors from males and females. Additionally, Figure 3-27 (p. 3-121) and Figure D-3 (p. D-31) appear to show combined data from males and females, although this is not stated. The information in the text about these figures and the figure legends should state which sex(es) are shown.

e. *The inhalation unit risk (IUR) was based on an occupational cohort by Gibb et al. 2020; 2015; 2000b) of chromate production workers at a facility in Baltimore, MD. Cox proportional hazard modeling of cumulative chromium exposure and lung cancer risk (with exposure lagged by 5 years) was used to estimate the POD at the exposure concentration that would cause a 1% extra risk of lung cancer in the U.S. population, resulting in an IUR for Cr(VI) of 2 × 10^{-2} (per µg Cr(VI)/m^3) (including application of ADAFs).*

The panel agreed that the available data for the inhalation unit risk (IUR) have been appropriately synthesized to describe the strengths and limitations and do support the conclusions presented. The search parameters are defined, and the process used to narrow the choice to one cohort was logical, rational, and carefully explained in detail. Further, the strengths and limitations of the top two choices (i.e., Baltimore, Maryland and Painesville, Ohio) for deriving the IUR are carefully and robustly delineated. Likewise, how the weight-of-evidence/quality-of-evidence decisions lead to the top choice (Baltimore, MD) for deriving the IUR were clearly described and scientifically justified. In addition, the dose-related decisions are transparent and scientifically justified, including study selection for dose-response analyses, point of departure (POD) estimates, modeling choices and assumptions, dosimetric adjustments, derivation of candidate values, and confidence in the calculated values. However, if upon additional evaluation, the evidence is sufficient to support a non-linear approach, this IUR and the ADAF would need to be amended accordingly.

Panel members raised some concerns about the key cohort studies (i.e., Gibb *et al.,* 2020, 2015, 2000b) that were used to derive the IUR as a large proportion of the cohort members were smokers and, as indicated in the draft IRIS assessment, 213 of the 217 lung cancer deaths that occurred in the study (98%) occurred among the smokers. This is a very high percentage that leads to speculation about the role of Cr(VI) and whether there could be an interaction between Cr(VI) exposure and smoking. The panel recognized that the analyses conducted by the EPA have attempted to adjust for smoking and that the possibility of an interaction is discussed in the draft IRIS assessment. The inclusion of studies where the prevalence of smoking is much lower would be beneficial. A recently published study by Behrens *et al.* (2023) provides a pooled analysis of 14 case-controlled studies from Europe and Canada evaluating exposure-response relationships for Cr(VI) and nickel in relation to lung cancer risk. It includes 16,901 lung-cancer cases and 20,965 controls. A measurement-based job-exposure-matrix (JEM) estimated job-year-region specific exposure levels to Cr(VI) and nickel, which were linked to the subjects' occupational histories. This JEM is described in detail by Peters *et al.* (2016). The average exposure in this study among controls is 40 µg/m^3-years (or 0.04 mg/m^3-years) among men and 26 µg/m^3-years among women, which is much lower than the exposures in the Painesville and Baltimore cohorts. Other aspects that make this study potentially informative are:

1) The study was controlled for smoking in pack-years and found exposure-response associations for never, former, and current smokers.
2) The analysis of the data was stratified for men and women, although the analysis of women was limited by the small sample size among those exposed. Among men, dose-response results appear to be consistent with results from the cohort studies, and EPA can confirm this. Among women, the findings were somewhat less consistent and not significant.
3) A cubic spline analysis was conducted to explore the shape of the dose-response and linear associations for Cr(VI) were observed. Adjustment for nickel exposure (the other chemical assessed in this manuscript) did not change the findings.

This study does however have limitations as acknowledged by the authors, particularly the exposure assessment, potentially resulting in differential bias which could have unpredictable effects on associations (either bias towards or away from the null).

It was also noted that Cr(VI) exposure has been found to increase cancer risks even among never smokers (Behrens *et al.*, 2023). Furthermore, the molecular characteristics of Cr(VI)-associated lung cancers were found to be very different in comparison to cancers caused by tobacco smoking. Cr(VI)-induced lung cancers were all squamous cell carcinomas (SCC) a majority of which (>80%) retained normal p53 (Kondo *et al.*, 1997) and displayed microsatellite instability (Hirose *et al.*, 2002). In contrast, a majority of lung SCC among smokers had mutated p53 (>80%) and were microsatellite stable (Cancer Genome 2012; Satpathy *et al.*, 2021).

Recommendation:

Tier 2:

- The panel recommends that studies be included where the prevalence of smoking is much lower (e.g., Behrens *et al.*, 2023) as it would be beneficial to strengthening the EPA's conclusions.

Supplemental Recommendations to Improve the IRIS Document

Several of the panel members had corrections or editorial comments that they felt would improve the draft IRIS assessment. These are presented in Appendix A and supplemental information in Appendix B.

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APPENDIX A – Editorial Comments

This appendix is a listing of comments that are primarily, but not exclusively, editorial in nature, and considered by the panel to be Tier 1 comments necessary to be addressed. They are listed in order, based on the charge question to which they pertain.

Charge Question #2

- Pages 3-21, lines 10-11. It is stated that four studies are included in Tables 3-4 and 3-5, but 5 studies are included.
- Table 3-12 (p. 3-65). In this table, the Ratio Measure (95% CI) for stomach cancer for each study is shown in **bold**. Since statistically significant data are often shown in bold, it was not clear that the bolding here indicated the data from stomach cancer, this likely to also be confusing to other readers. The information associated with the table should be revised to clearly indicate that the stomach data, not statistically significant data, are highlighted.
- Pages 3-70, lines 9-12. The sentence starting with "As noted in Table C-43…" needs clarification. It mentions "two industry groupings with higher certainty of Cr(VI) prevalence…" but it appears that more than two industry groupings are then mentioned.
- Pages 3-80, lines 1-5. It should specifically mention that none of the 23 human genotoxicity studies listed in Table 3-17 evaluated gene mutations.
- In cases where studies were considered uninformative (e.g., p. 3-20), adding a few sentences summarizing why the studies were considered uninformative would be helpful because it will allow the reader to determine the consistency of decision-making throughout the draft IRIS assessment without having to go to the external HERO or HAWC databases.
- Uninformative studies appear to be displayed in tables for animal studies (e.g., Table 3-6) but not for epidemiological studies (e.g., Table 3-4). EPA should review the draft IRIS assessment for opportunities to present animal and epidemiological literature in a more consistent fashion.

Charge Question 3b.ii.

- The EPA needs to correct the text (p. 3-33 to 3-34) stating that "laboratory animals were exposed to aqueous aerosols of Cr(VI)…thus the effects observed…were unrelated to particle responses." This statement is unclear.
- The EPA should include a comment in the statement (p. 3-39, line 10) that the observed changes in histiocytosis were biologically significant.
- The EPA should correct and clarify some reporting issues in the paragraph on modeling (p. 4-38, lines 9-22 of the draft IRIS assessment) regarding exposure concentration units and the density of sodium dichromate aerosols.
- P3-34, line 32: Cr(VI) is not a potent oxidizer at physiological pH – rather it is a selective oxidizer toward ascorbate primarily and thiols secondarily. There is also a similar inaccurate reference to Cr(VI) as "a strong oxidizer" elsewhere in the IRIS draft.
- p. XVIII, line 10 *and many other places: "…lower respiratory toxicity. EPA determined that Cr(VI) is likely to cause lower respiratory toxicity…".* It is an imprecise/confusing description of respiratory toxicity. Change to: lower respiratory tract toxicity.

- p. XIX, lines 9-11: "*Effects of Cr(VI) on the nasal cavity have been well established to occur in humans, and this was also the most sensitive effect. It is considered protective of the other noncancer effects."* It is a confusing statement: How can nasal septum damage/ulceration protect against other forms of tissue damage?
- Discussion of the role of p53 in apoptotic responses (p3-36) would benefit from the inclusion of information on a limited p53 activation by Cr(VI) *in vivo* and ascorbate-restored human cells (Luczak MW *et al.*. 2019 and refs therein, PMID: 31388677).
- Fig. 3-7: There is no evidence that ascorbate acts as a stabilizing agent for Cr(V) and/or Cr(IV) generated during Cr(VI) reduction by thiols.

Charge Question 3d.ii

- The EPA should not use the phrase "non-statistically significant increases" (p. 3-165, line 15) as it is inappropriate. A parameter cannot be said to increase if it does not significantly differ from the control.
- The EPA should consider adding an appropriate legend to Figure 3-21 (p. 3-175). Unlike similar figures, this figure does not contain a legend describing the meaning of markers (i.e., black dots, red and blue triangles).

Charge Question 4

- The complete results of the BMD modeling should be included in the draft IRIS assessment Appendix material as a separate chapter.
- Because there are orders of magnitude differences in the PODs derived using different models that have rather similar statistical parameters, it is challenging to evaluate the selection of "the lowest Akaike's information criterion (AIC)/highest p-value" approach to POD selection without reviewing the complete BMD modeling results, which are needed to determine how well the model fits the data. To improve transparency, the results for the model that was selected as the basis for the POD should also be shown in the main draft IRIS assessment.
- Additionally, the type of POD (BMD, NOAEL, or LOAEL) for each endpoint that are shown in Tables 4-3 and 4-11 should also be included in Figures 4-3 and 4-7.
- Table 4-3 (p. 4-12): The information provided in some of the columns is unclear, and the table headings for those columns should be clarified. Specifically, units (mg/kg-d) should be added to the "$BW^{3/4}$ adjust" column, since it is not clear (although stated in the footnote) that this column provides the dose after the internal dose is adjusted for the ratio of human to animal body weight to the ¼ power.
- For further clarification, adding another column between "TWA BW" and $BW^{3/4}$ adjust" that shows the $BW^{3/4}$ scaling factor for each endpoint would be useful. Also, "TWA" should be defined.
- p. 4-14, lines 22-30. Line 25 states that "a PBPK model or $BW^{3/4}$ scaling was used to convert doses in rodents to equivalent doses in humans." However, both the PBPK model and $BW^{3/4}$ scaling were used to derive HEDs from the doses administered to rodents, and it is suggested the text be revised to reflect this. Similarly, on lines 29-30, suggest revising "any residual pharmacokinetic uncertainty not accounted for by the PBPK model" to "any residual pharmacokinetic uncertainty not accounted for the by the PBPK model and $BW^{3/4}$ scaling."

Charge Question #5a

- On p. 4-14, line 25, "a PBPK model or $BW^{3/4}$ scaling" should be changed to "a PBPK model and/or $BW^{3/4}$ scaling."
- Although standard practice, the panel suggests that the routine use of an UF of 3 to represent 10^0.5, or half an order of magnitude between 1 and 10, should be described when the UFs are first introduced on page 4-14.
- Amend Figures 4-3 and 4-7 to (i) indicate with different symbols different types of PODs used (BMC, NOAEL, or LOAEL) and (ii) clarify the contribution of each UF by splitting the gray bar into shorter segments signifying each UF applied (and use colors or shading to distinguish among UFs).
- Regarding Appendix D, Tables D-23 and D-24, p. D-29 – D-30, the results of both approaches for uncertainty factor application (i.e., the approach that was selected and the alternative approach) should be included in these tables so the reader can compare the results of the two approaches.

Charge Question #5b

- The EPA should specifically mention that the lower 1% value was used in the selection of this UF on p. 4-14, lines 11-21. On Page 4-14, Lines 11-21, the draft IRIS assessment states that "the pharmacokinetic component of this factor may be replaced when a PK model is available that incorporates the best available information on variability in pharmacokinetic disposition in the human population (including sensitive populations)." Yet, the charge question refers to "susceptible populations." The panel was unclear on the distinction between "sensitive" and "susceptible" populations.

Charge Question #5c

- Tables 4-11 and 4-12, which present information relevant to this question, should use consistent units (mg/m^3 or $\mu g/m^3$).

Charge Question 6a

***Modify Table 3-19: "Prioritized genotoxicity studies in animals exposed to Cr(VI)"* with more accurate comments about the data presented in it.**

- Page 3-97, the row describing "Thompson *et al.*, (2015b) Low confidence," the third column summarizes the data as "No effect on levels of γH2AX." Indeed, the authors made this claim. However, in the 4th column, labeled, "comments", it needs to state that the paper does not provide any quantification of gamma-H2A.X. There is only a couple of pictures and the statement that there was no effect, but there are no actual reported values provided for levels of gamma H2A.X in controls or in treated groups so no means to understand the magnitude of the levels or the statistical relationship. Also, it should indicate the pictures are from a single animal.

- Page 3-110, lines 8-10: "*However, other genotoxicity endpoints from in vivo oral exposure studies specific to GI tissues were negative, including γH2AX, a marker of DNA double-strand breaks (Thompson et al.., 2015b; Thompson et al.., 2015a),…*" This comment is overstated as Thompson *et al.*., 2015b, as noted above, did not provide any gamma-H2A.X levels so it cannot be ascertained if this claim is correct and Thompson *et al.*., 2015b has such high background levels in its crypt negative control samples, the data are uninterpretable. This comment needs to be deleted or modified to make the uncertainty much clearer.
- Page 3-121, lines 12-14: "*After 13 weeks of exposure, Thompson et al. (2015a) detected a weak Cr signal (0.4 µg/g) in the 24 small intestine crypts that were examined, with a 35- fold higher (14 µg/g) mean concentration in the villi.*" The detection limit of the instrument used was 0.15 ug/g, therefore the Cr level in the crypts was 2.7-fold higher than the detection limit. Moreover, the instrument is relatively insensitive to Cr as other techniques would have offered detection limits of 0.001 ug/g, for which a level of 0.4 would have been 400-times higher than background. Accordingly, the assessment of the Cr signal as "weak" would appear to be a function of the lack of sensitivity of the instrument. The word "weak" should be deleted and the number allowed to stand alone as it was a measurable level of Cr.
- Page 3-121, lines 12-15: "*After 13 weeks of exposure, Thompson et al. (2015a) detected a weak Cr signal (0.4 µg/g) in the 24 small intestine crypts that were examined, with a 35- fold higher (14 µg/g) mean concentration in the villi. A separate 7-day study reported the absence of Cr in the crypt compartment without quantitative results*…'. Both studies only considered Cr levels in a single animal, when numerous animals were available. This increases the uncertainty and should be noted.

Avoid including data/conclusions from cell lines unable to measure the effect **considered:**

- Page 3-37 lines 1-27, there is mention of p53 driven results in MOLT-4 cells (lines 2-8). The MOLT-4 cell line carries a p53 mutation, although there are reports using "MOLT-4" cell lines, where the cell line is not actually MOLT-4 but has been cross contaminated with another cell line altering its P53 status. These MOLT-4 outcomes should not be included here in a discussion of normal P53 function due to the expectation of a P53 mutation in the cells rendering them not a normal p53 model. This exclusion does not change the overall point of the passage.
- Page 3-37, lines 1-27, there is mention of p53 driven results in BEAS2B cells (lines 2-8). The BEAS-2B cells were immortalized with the large T antigen simian virus 40 (SV40). Large T-antigen immortalizes cells by binding to the p53 and the retinoblastoma (Rb) proteins rendering them functionally inactive. Consequently, it is inappropriate to assess normal p53 function in BEAS-2B cells as p53 is nonfunctional in them. These BEAS-2B outcomes should not be included here in a discussion of normal P53 function. This exclusion does not change the overall point of the passage.

Discuss the uncertainty in cell culture studies as was done for the human and **animal studies:**

- In section 3.2.2.3. Mechanistic Evidence, page 3-55, lines 1-21, there is a discussion of Thompson *et al.* 2012a, which claims to report cytotoxic effects and that DNA double strand breaks measured as gamma-H2A.X were only present at cytotoxic levels. However, careful inspection shows that this study did not actually measure cytotoxicity. In fact, they measured

a reduction in cell number, which on its surface could be construed as cytotoxicity, but it could also simply be growth arrest induced by the presence of the DNA double strand breaks. Thus, one cannot ascertain from this study whether cells actually died or simply arrested in their growth cycle, repaired the breaks and resumed growth. The conclusion of cytotoxicity by the study is incorrect. One can only conclude there were fewer cells, which could be cell death or could be growth arrest. Cr(VI) is known to induce a G2/M growth arrest which could easily explain the results and this uncertainty should be discussed.
This alternative interpretation has significant implications for the conclusions of the paper as "…differentiated cells were more resistant to chemical-induced cytotoxicity…" becomes "that differentiated cells were more resistant to chemical-induced growth arrest".

- In that same section, the study used Caco-2 cells, which are a tumor cell line that has an unstable phenotype with some versions considered p53 null and others containing a p53 mutation. They also have 96 chromosomes instead of the normal 46. These aspects can confound the interpretation and should be discussed.
- Page 3-36, lines 34-38, there is mention of 10 published studies of "Cytotoxicity occurring at micromolar Cr(VI) levels that increases with dose and duration of 34 exposure…". There are more studies that consider this outcome in human lung cells that are not included. The document has been careful to clarify why particular subsets of studies have been chosen but did not do the same here. The document should clarify why these 10 were chosen as representative over others.

***Discuss the uncertainty and limitations in gamma-H2A.X studies as was done for* other experimental endpoints:**

- Page 3-110, lines 6-13, in the presentation/discussion of Thompson *et al.* 2015a and 2015b, there is no mention of the limitation of the gamma-H2A.X analysis. The assay used is less sensitive than the typical approach of measuring gamma-H2A.X by immunofluorescence on a confocal microscope (used by this group in their cell culture study). It is further limited because they did not measure immunofluorescent foci, which is the more rigorous measure. The assay design here lends to uncertainty in the negative finding and the absence of clear quantified data on a per cell basis further increases the uncertainty of the reported negative outcome. This uncertainty should be included and discussed.
- In section 3.2.2.3. Mechanistic Evidence, page 3-55, lines 1-21, there is a discussion of Thompson et al. 2012a, which purports to quantify gamma-H2A.X foci, as most of the Cr(VI) literature has done and went with a more insensitive measure of total gamma-H2A.X expression and that should be commented on as well. It is also notable that the authors actually measured total gamma-H2A.X by immunofluorescence (Figure 4) and consequently, quantifying the foci would have been as straight forward as increasing the magnification and counting. Inspection of Figure 4 as presented actually shows no foci at what is considered a high, positive dose of Cr(VI), which raises concern about the technical quality of the stain and that it may not be working properly to accurately measure gamma- H2A.X. It could be a magnification issue in the picture, but the absence of any foci at all and the excessive staining suggests it is a technical issue. Regardless, this approach creates uncertainty in the interpretation of the data. This uncertainty should be included and discussed.

Other edits

- In section 3.2.3.3. Genotoxicity Evidence (All Routes), pages 3-89, lines 13-14: The introduction to the passage reads (italics and bold red font added here to improve visibility): "*Among the 16 studies evaluating micronuclei, four were rated as medium confidence and **12** were rated as low confidence.*" What follows is a discussion of the four rated as medium confidence followed by a paragraph dedicated to the set of the ones rated as low confidence. There is a disagreement at the start of that following paragraph (lines 23-24), as it states: "*Among the **11** low confidence studies, there were ten that reported increased micronuclei for at least one cell type.*" The apparent number contradiction should be resolved as to whether there 11 or 12 low confidence studies.
- Page 3-51, lines 23-24: The passage reads: "*Two follow-up publications using the same experimental subchronic dataset in female 23 B6C3F1 mice (Thompson et al., 2011) reported increases in some markers of duodenal villus…*" The challenge is that it sounds like Thompson *et al.* is one of the two follow-ups and it is not. Consider changing to: "*Two follow-up publications using the same experimental subchronic dataset in female 23 B6C3F1 mice as Thompson et al., (2011) reported increases in some markers of duodenal villus…*"
- Page 3-132, lines 29-31: The passage reads: "*As discussed in Section 3.2.2.3, some discrepancies have been noted, including the lack of increased mitotic activity in hyperplastic duodenal crypt cells in mice (Thompson et al., 2015b; O'Brien et al., 2013), although **follow-up** analysis of the mice exposed via drinking water for 7 and 90 days (Thompson et al., 2011) reported a significant…*" It is unclear how a study published in 2011 can be a "follow-up" for studies published in 2013 and 2105. The wording should be adjusted.
- 8-OHdG does not appear to be defined as 8-hydroxy-2′-deoxyguanosine in the document and should be defined and added to the abbreviation list.

<u>Charge Question #6d</u>

- Figure 4-8, p. 4-52. In the figure key, the dotted line labeled "linear extrapolation" should be labelled "95th percent upper confidence limit for response at BMD." Also, the model that was selected for each endpoint should be stated.
- Table 4-13, p. 4-53. In the Model column, "1^{o} MS" should be defined, since the reader may not know what this stands for. Also, the "BMR" column should say that the BMR is 0.1 or 10%, not just "10." Additionally, the information in the two rows in the Extrapolation Model column for tumors in the mouse small intestine should be shown as "PK and BW$^{3/4}$" and "BW$^{3/4}$" rather than "PK" and "BW$^{3/4}$."
- Section 4.3.3, p. 4-53, lines 15-19. It should be stated that the tumor incidence selected for derivation of the CSF is 0.1 (10%). This value is used in the equation shown at the end of line 16, but it is not stated previously that this was the incidence that was selected.
- The panel recommends that the discussion about the application of ADAFs to adjust the slope factor of 0.3 (per mg/kg-d) to 0.5 (per mg/kg-d) be revised to state that the slope factor of 0.3 (per mg/kg-day) applies to risks from less-than-lifetime exposures that occur only in adulthood, such as occupational exposures.

- The panel recommends that the text be revised to clearly indicate that the modeling of the tumors in males as well as the modeling of the tumors in females resulted in the same slope factor.
- The panel recommends that the information in the text about Figure 3-27 (p. 3-121) and Figure D-3 (p. D-31) be clarified and the figure legends should state which sex(es) are shown as they appear to show combined data from males and females.

Appendices

- Appendix C, Table C-10. Relevant to the point mentioned above, the information in the "Added Uncertainty" column for "$BW^{3/4}$-adjusted unreduced Cr(VI) dose - Daily mg Cr(VI) emptying from the stomach, per kg BW, multiplied by $(BWa/BWh)^{0.25}$" in the last row of Table C-10 on p. C-18 needs clarification or revision. It is stated that this approach "does not incorporate volume of gastrointestinal tissue, a site of observed toxicity." However, volume of gastrointestinal tissue is accounted for since, as above, "interspecies scaling by $BW^{3/4}$ is numerically similar to scaling by small intestinal volume."
- Appendix C, Table C-0, p. C-13. It appears that the Media (species) column for the second row of entry for Kirman *et al.* (2016) about gastric fluid reanalysis should say "mouse, rat" rather than "rat, mouse, human) since only mouse and rat data are shown in the Findings column.
- Appendix D of the draft IRIS assessment, p D-8, lines 5-7. For clarification, the panel suggests stating which specific criteria led to the conclusion that there was too much uncertainty in the BMD estimates for diffuse epithelial hyperplasia in mice to use the model results to determine POD.
- Appendix D, of the draft IRIS assessment, Table D-15. Footnote c is missing and should be added.
- Appendix D of the draft IRIS assessment, Tables D-21 and D-22, p. D-26 – D-27. The panel suggests including results of the approach that includes both PBPK adjustment and BW3/4 scaling along with the results of the default BW3/4 scaling so the reader can compare the results of the two approaches.
- Appendix D of the draft IRIS assessment, p. D-25. The panel suggests stating in the text that the first order multistage model was selected for use as the basis for the CSF for all tumor types (as shown in Table 4-13).

APPENDIX B – Detailed Comments

The following information consists of highly detailed comments made by one or two panel members that the EPA may wish to consider as they revise the draft IRIS Toxicological Review of Hexavalent Chromium. Comments of individual panel members are indicated as such. In some cases, summary text is provided for context to the panel's deliberations.

This Appendix is organized by Charge Question to facilitate cross-referencing with the Committee's consensus responses to the charge questions that are provided above.

Charge Question 1:

Individual committee members provided the following references which the EPA may consider when revising the draft IRIS assessment.

Cr(VI) MOA Studies

1. Bhat, VS, Cohen, SM, Gordon, EB, Wood, CE, Cullen, JM, Harris, MA, Proctor, DM, and Thompson, CM, 2020. "An adverse outcome pathway for small intestinal tumors in mice involving chronic cytotoxicity and regenerative hyperplasia: a case study with hexavalent chromium, captan, and folpet." *Crit. Rev. Toxicol.* 50(8):685-706.
2. Chappell, GA, Wikoff, DS, and Thompson, CM, 2021a. "Assessment of mechanistic data for hexavalent chromium-induced rodent intestinal cancer using the key characteristics of carcinogens." Toxicol. Sci. 180: 38 – 50.
3. Chappell, GA, Wolf, JC, and Thompson, CM, 2021b. "Crypt and villus transcriptomic responses in mouse small intestine following oral exposure to hexavalent chromium." *Toxicol. Sci.* 186: 43 – 57.
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Costa Laboratory

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9. Coogan, T.P., Motz, J., Snyder, C.A., Squibb, K.S., and **Costa, M.** Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure to rats to potassium chromate. *Toxicol. Appl. Pharmacol.***109**:60-72 (1991).
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Charge Question #2

A majority of the panel felt that EPA's determination of low quality for the oral genotoxicity studies was supported, and that the agency adequately supported this determination (in addition to and unrelated to the lack of a maximum tolerated dose (MTD)). For example, regarding Thompson *et al.* (2015b), Table 3-19 of the draft IRIS assessment states that the baseline incidence of micronuclei has not been established for the tissues that were evaluated and that the number of cells analyzed was insufficient to detect a change in the number of micronuclei. It also should be noted that a minority of the panel expressed concern in the low confidence ratings given to the oral *in vivo* genotoxicity studies (see table 3-19), due of lack of an MTD and higher confidence ratings given to the positive genotoxicity studies using *in vitro* assays and intraperitoneal (ip) injection (and hence EPA's choice of a mutagenic MOA, see response under CQ6 for further discussion). These individuals noted that while *in vitro* and ip studies

are potentially relevant for hazard identification, they are of limited relevance for dose-response assessment for oral exposures to Cr(VI). They concluded that the relationship between the genotoxicity indicator and tumor production is more important, specifically whether relevant genotoxicity findings are found prior to tumor formation, both in terms of dose and temporality. These individuals concluded that other concerns with the oral genotoxicity studies (e.g., nonpositive micronuclei findings from the dimethylhydrazine control) were appropriately addressed by considering the positive findings with cyclophosamide in Thompson *et al.*, 2015. Another example regarding study quality, e.g. whether doses in Thompson *et al.*, 2015 were cytotoxic, were considered by these individuals to have also been adequately addressed (as discussed in the public comments summary table by Thompson and Wikoff 2023). These individuals recommended that the low confidence ranking for the oral genotoxicity studies based on the lack of an MTD and other concerns regarding methodology and interpretation of the studies should be reconsidered by EPA.

Charge Question #3

CQ3a-i: Gastrointestinal

One panel member noted the following:
To help prevent misinterpretation or an overly broad interpretation of the phrase, "given sufficient exposure conditions" in this context means that given the indeterminate human evidence but robust laboratory animal evidence for gastrointestinal effects (as characterized in Table 3-10), sufficiently high exposure over a sufficiently long duration will likely produce gastrointestinal effects (noncancer) in humans at some point as dose and duration rise. However, it will not necessarily produce the same effects at the same doses/lowest-observed-adverse-effect-levels (LOAELs) that caused such effects in laboratory animals when extrapolated to estimated human equivalent doses (HEDs). This is due to the uncertainty associated with these extrapolations and the potential interspecies toxicodynamic differences relative to the most sensitive laboratory animal species.

CQ3a-ii: Gastrointestinal

One panel member expressed the following concerns about the choice of species for deriving the POD and overall RfD, stating:

a. The overall RfD has not been fully scientifically justified regarding species selection. Table 4-4 (p. 4-16 to 4-17) illustrates these key decision points (i.e., the most appropriate animal model(s) to use for humans) but no justification was provided. Most specifically, gastrointestinal effects in humans are assumed to have a dose-response (or POD at a minimum) similar to female mice (i.e., female mouse dose-response data, with dosimetric adjustments, are being used as the best laboratory animal surrogate data to represent dose-response in humans), but humans are assumed to have a dose-response like the rat for liver effects, a dose-response like mice for developmental effects, and finally humans are assumed to have a dose-response for hematological effects similar to rats, all without scientific justification for why the species ultimately providing the basis of the toxicity factor is

expected to have a dose-response more representative of that in humans than other species for which data are available.

b. Regarding laboratory animal models and the gastrointestinal tract, comparing the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, Kararli (1995) concluded that while data indicate that no single animal can mimic the gastrointestinal characteristics of humans (i.e., human studies cannot be substituted by animals), the selection of the right animal model for a given purpose is possible. This panel member did not agree that it was scientifically robust to simply state, "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and this statement could be equally applied to negative findings in a given laboratory animal species. Another panel member, however, noted that this approach does indeed reflect standard EPA policy and is intended to err on the side of protecting public health.

c. The same panel member also noted that when the issue of applicability of animal data to humans is not addressed scientifically, which is relatively common with few exceptions (e.g., male rat alpha 2μ-globulin nephropathy), use of data from a given animal species for dose-response assessment applied to humans can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species) that pertains directly to the meaningfulness of the resultant toxicity factor itself and for credibly informing risk/hazard management decisions.

d. The same panel member recommended that the EPA explicitly acknowledge this uncertainty. The National Research Council (NRC) has advised that proper characterization of uncertainty is essential in risk assessment as an assessment that omits or underestimates uncertainty can leave decision-makers with a false sense of confidence in estimates of risk (NRC 1983, 1994, 1996, 2002). Use of an animal model as a surrogate for humans is an aspect of uncertainty that should be adequately addressed and characterized in an assessment (EPA 2005a). However, the limited uncertainty section discussions of: (1) the possibility that the observed effects in mice may be exhibited in different sections of the alimentary tract in humans (e.g., the oral cavity, esophagus, and stomach) (Section 4.1.6.1), and (2) uncertainty related to dose-response modeling (Section 4.1.6.6), do not address the larger uncertainty regarding the most appropriate animal model and whether a similar dose-response may or may not be expected in humans (after appropriate dosimetric adjustments). The panel member who expressed these opinions further emphasized that the choice of animal model consequently reflects policy rather than scientific justification. Other panel members, however, did not express any concerns about this approach as it reflects EPA's public health protective positions.

In the view of one panel member, assuming comments on the lack of a full scientific justification for use of gastrointestinal effects data from female mice are addressed, the LOAEL-based POD_{HED} of 0.0911 mg/kg-d for diffuse epithelial hyperplasia of the duodenum appears sufficiently conservative for regulatory use in deriving an osRfD (e.g., minimally adverse effect,

most sensitive species, most sensitive sex, conservative pharmacokinetic (PK) adjustment). For example, when the LOAEL-based POD_{HED} is divided by the EPA UF_L of 10 to make a more appropriate comparison to BMDL-based POD_{HED} values (for which a UF_L is not used/justified), the resulting POD_{HED} (0.00911 mg/kg-d) is approximately 5 times lower than the BMDL-based POD_{HED} based on male mice (0.0443 mg/kg-d; Table 4-3). As a different and potentially equally-relevant animal model, no such comparisons are possible for the rat because none of the gastrointestinal tract effects in Table 3-50 (p. 3-314) were carried forward for dose-response modeling (see Table D-1) because such effects were observed less consistently and at higher doses in rats compared to mice (p. 4-4). However, regarding use of the LOAEL as the POD because there was "too much uncertainty in estimating the BMDL" (p. 4-10, line 11), the panel noted that EPA's BMDS recommended the log-logistic model as a viable model (p. 9 of the pdf of EPA's model log files, EPA 2021) despite that the BMD_{10} was ≈4-fold lower than the lowest dose. This recommended model provided a $BMDL_{10}$ of 0.052983 mg/kg-day, which is appreciably lower than that for male mice (0.121 mg/kg-day; Table 4-3). It may be even more uncertain to simply apply a default UF_L of 10 to the LOAEL (0.302 mg/kg-day). The resulting value (0.0302 mg/kg-day) is lower than the $BMDL_{10}$ (0.052983 mg/kg-day) that is at least informed by the fit of a model (log-logistic), and one that is considered viable and recommended by EPA's BMDS.

The LOAEL (in units of mg/kg-d Cr(VI)) was converted to an internal dose using the PBPK model. Although it should not generally be considered as good of a dose metric as absorbed target tissue dose, which is more closely (i.e., causatively) associated with the toxic effect and indisputably internal, the "internal" dose used by EPA was the average rodent dose escaping reduction (really an adjusted applied dose in mg/kg-d) multiplied by $(BW_A/BW_H)^{1/4}$. The adult-based human PBPK model was used to estimate the daily mg/kg Cr(VI) dose that must be ingested to achieve this same internal dose. To account for interindividual variability, the HED was determined by Monte Carlo analysis using the lower 1% value of 20,000 Monte Carlo PK simulations needed to achieve the internal dose POD (i.e., 0.0911 mg/kg-d in Figure D-9 on p. D-47). This is a conservative choice for this admittedly minimally adverse effect (Section 4.1.2.2, p. 4-10).

One panel member recommended use of a different modeling approach and explained their concerns as follows. First, regarding choice of dose metric for BMD modeling, diffuse epithelial hyperplasia of the duodenum in female mice (NTP 2008) was modeled by Haney (2015c) on the basis of duodenum tissue absorbed dose (mean added mg Cr/kg tissue at the same drinking water concentrations; Kirman *et al.* 2012). The Log-Logistic and Dichotomous-Hill models provided adequate fit to the mouse data (Table 5 of Haney, 2015c) with a goodness-of-fit p value > 0.1 and scaled residuals <|2|, fit confirmed by visual inspection. These models provided almost identical fits (Figure 4 of Haney, 2015c) with the lowest AIC value, highest goodness-of-fit p value, the same BMD_{10} values and very similar $BMDL_{10}$ values (Table 6 of Haney, 2015c). The $BMDL_{10}$ values showed good agreement with their corresponding BMD_{10} values, all being within a factor of 1.5. Furthermore, the $BMDL_{10}$ values using the mean value of additional mg Cr/kg tissue as the internal target tissue dose metric were very similar to those using the 95% lower confidence limit of tissue concentrations (i.e., within a factor of 1.2), increasing confidence in use of these results. Unlike EPA BMD modeling results in Table D-5, the Log-Probit model had a $BMDL_{10}$ within a factor of 3 of the Log-Logistic and Dichotomous-Hill

models, which had the lowest AIC. This BMD modeling approach: (1) did not exhibit the same problem as EPA's results (i.e., significantly different BMDs/BMDLs with BMDLs for the full dataset differing by as much as 27-fold; see p. 4-10 lines 13-16 and Table D-5); (2) meets all EPA modeling criteria in Section D.1.1 (on p. D-7); (3) is an approach that considers all the data and is generally considered more scientifically sophisticated than the NOAEL/LOAEL approach; and (4) is based on a dose metric more closely causally related to the toxic effect, target tissue absorbed dose. One panel member recommended that the EPA should: (1) attempt to scientifically justify whether the mouse or rat is likely most biologically representative of humans such that the same or similar effects (gastrointestinal, etc.) are expected in humans at similar doses when converted to human equivalent doses (HEDs); and (2) in the event (1) cannot be established with sufficient scientific confidence, acknowledge within the assessment that the choice of the most appropriate laboratory animal model for prediction of Cr(VI)-induced adverse effects in humans has not been scientifically established (i.e., is not "settled science") but rather species selection is based on policy.

CQ3b: Respiratory (noncancer outside of nasal cavity)

One panel member reasoned that the total UF of 1,000 for deriving the osRfC may be reduced to 300 following a more detailed reconsideration of the UF_D value. Regardless of whether the total UF value changes, a more detailed discussion would better scientifically justify the value and this osRfC. As with the RfD, this justification of the animal model utilized for surrogate human dose-response data is a major area of uncertainty that EPA does not attempt to scientifically address in the draft assessment. It is not scientifically robust to simply state, "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and this statement could be equally applied to negative findings in a given laboratory animal species. Between this and a total UF of 1,000, uncertainty spans several orders of magnitude, and it seems difficult to state with confidence that this osRfC meets the definition of an RfC (i.e., with uncertainty perhaps spanning an order of magnitude). This is not to suggest that whether an RfC (or RfD) has uncertainty that spans no more than an order of magnitude can be determined simply by whether the total UF is greater than 10, because for example, some UFs reflect that the POD must be downwardly adjusted to some extent to attempt to account for a known factor (e.g., in the absence of a NOAEL, it is known with certainty that a $UF_L > 1$ is needed to adjust the LOAEL to some sort of surrogate NOAEL value). Rather, this comment is meant to recognize that as a POD, which can be associated with its own uncertainties (e.g., due to interspecies differences in sensitivity), is divided by progressively greater-and-greater total UFs (up to 3,000), leading to lower confidence in the resulting value (RfC or RfD) approximating a safe human dose (likely without appreciable risk of effects) within an order of magnitude of imprecision (± a factor of 3.16; p. 1-15 of EPA 1994) of the safe dose that would be established if an accurate general population threshold (or "true" biological threshold as termed on p. 1-4 of EPA 1994) for adverse effects were known (i.e., there is less confidence that the RfC or RfD meets its definition). Moreover, sufficient chronic human data would affect every UF used to derive this value. Accordingly, concludes one panel member, there can be greater confidence in the RfC/RfD value (i.e., with uncertainty perhaps spanning an order of magnitude) when the POD is based on a sensitive human subpopulation with a low total UF (perhaps even as low as 1), as compared to when the POD is based on a laboratory animal where there are significant

interspecies differences in sensitivity, and the total UF is significantly higher (perhaps several orders of magnitude).

CQ3d: Hepatic

One panel member noted that the osRfD for hepatic effects was based on the lowest candidate toxicity value, which was based on chronic inflammation in female F344 rats reported in NTP (2008). Histological changes were less severe in male rats and mice; therefore, female rats may be the most sensitive group (Section 4.1.4.2, p. 4-18). Like the osRfD for gastrointestinal effects in female mice, EPA has not attempted to scientifically justify that the animal model utilized (rat) is a better laboratory animal model than alternatives (mouse) for the same or similar hepatic effects in humans. Yet, contrary to that assumed for gastrointestinal effects, for hepatic effects, humans are assumed to have a dose-response (or POD at a minimum) like female rats (i.e., female rat dose-response data, with dosimetric adjustments, are being used as the best laboratory animal surrogate data to represent dose-response in humans).

When data applicability to humans is not addressed scientifically, use of data from a given animal species for dose-response assessment applied to humans can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species) that pertains directly to the meaningfulness of the resultant toxicity factor itself and for credibly informing risk/hazard management decisions. It is not scientifically robust to simply state, "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and this statement could be equally applied to negative findings in a given laboratory animal species. The limited uncertainty section (Section 4.1.6) does not address this larger uncertainty regarding the most appropriate animal model and whether a similar dose-response may or may not be expected in humans (after appropriate dosimetric adjustments). This is a major area of uncertainty, but it is not without precedent that it be explicitly acknowledged in the associated uncertainty section (e.g., EPA 2021). Furthermore, it can be a significant uncertainty, that when considered amongst others can preclude a judgment, that an osRfD meets the definition of an RfD (i.e., with uncertainty perhaps spanning an order of magnitude) notes one panel member.

As an example of the importance of this issue for hepatic effects notes one panel member, the candidate osRfD values for chronic liver inflammation (the selected critical effect in rats) differ by a factor of 27 between the female mouse (0.0182 mg/kg-d) and female rat (0.000669 mg/kg-d) (Table 4-4), with the mouse osRfD incorporating BMD modeling (as opposed to the LOAEL-based POD for the female rat) and 10-fold lower total uncertainty (10 for the female mouse osRfD versus 100 for the female rat osRfD). Thus, the mouse-based osRfD derived using BMD modeling and a 10-fold lower total UF may be viewed as having less scientific uncertainty and greater scientific confidence. Recognizing and considering the magnitude of the uncertainties associated with the selected rat-based osRfD, it may be a stretch to state that "there is high confidence in this osRfD" (p. 4-18) if this is meant to imply either that uncertainty spans no more than "an order of magnitude" or that the value "is not likely to change substantially as more data become available" (EPA 1994; pp. 1-7 and 1-15).

For example, notes one panel member, having sufficient human data or data supporting the mouse as a better laboratory animal model, the value could / would change substantially. Without scientific justification for which animal model is expected to be the most predictive laboratory surrogate for the human dose-response (suggested above), it appears that policy (as opposed to scientific considerations) drives the selection of the osRfD and that it is unknown whether the osRfD for hepatic effects meets the definition of an RfD (i.e., with uncertainty perhaps spanning an order of magnitude) due to the magnitude of associated uncertainties (e.g., significant interspecies differences compounded by a two order of magnitude total UF). This is not to suggest that whether an RfD (or RfC) has uncertainty that spans no more than an order of magnitude can be determined simply by whether the total UF is greater than 10, because for example, some UFs reflect that the POD must be downwardly adjusted to some extent to attempt to account for a known factor (e.g., in the absence of a NOAEL, it is known with certainty than a $UF_L > 1$ is needed to adjust the LOAEL to some sort of surrogate NOAEL value). Rather, this comment is meant to recognize that as a POD, which can be associated with its own uncertainties (e.g., due to interspecies differences in sensitivity), is divided by progressively greater-and-greater total UFs (up to 3,000), less confidence is associated with the resulting value (RfD or RfC) to approximate a safe human dose (likely without appreciable risk of effects) within an order of magnitude of imprecision (± a factor of 3.16; p. 1-15 EPA 1994) of the safe dose that would be established if an accurate general population threshold (or "true" biological threshold as termed on p. 1-4 of EPA 1994) for adverse effects were known (i.e., there is less confidence that the RfD or RfC meets its definition). Moreover, sufficient chronic human data would affect every UF used to derive this value. Accordingly, there can be greater confidence that an RfD/RfC meets the definition (i.e., with uncertainty perhaps spanning an order of magnitude) when the POD is based on a sensitive human subpopulation with a low total UF (perhaps even as low as 1), as compared to when the POD is based on a laboratory animal, there are significant interspecies differences in sensitivity, and the total UF is significantly higher (perhaps several orders of magnitude). This one panel member recommends that the EPA should: (1) attempt to scientifically justify whether the rat or mouse is likely most biologically representative of humans such that the same or similar hepatic effects are expected in humans at similar doses when converted to HEDs; and (2) in the event (1) cannot be established with sufficient scientific confidence, acknowledge within the assessment that the choice of the most appropriate laboratory animal model for prediction of Cr(VI)-induced adverse hepatic effects in humans has not been scientifically established (i.e., is not "settled science") but rather species selection is based on policy.

CQ3e: Developmental

One panel member noted that taken together, the weight of the available scientific information presented reasonably supports that assuming sufficiently high exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions" as stated in Table 3-47), Cr(VI) exposure is likely to cause developmental effects in the general human population, which includes potentially susceptible subpopulations. The bases for this weight-of-evidence decision are clearly described in the text (e.g., *Integration of Evidence* on p. 3-299 and 3-300) and Table 3-47 of the draft IRIS assessment. This agrees with the panel consensus viewpoint.

The EPA (p. 3-284) described the results of two studies using geographically-based measures of exposure that "examined associations based on proximity to a Cr(VI) contaminated site (kilometers from center of polluted area in Eizaguirre-García *et al.* (2000), primarily affected town vs. rest of county in Remy *et al.* (2017). The developmental effects examined in these studies included spontaneous abortion, early pregnancy loss (not defined), pregnancy complications, and infant health (Remy *et al.*, 2017) and congenital malformations/anomalies (Remy *et al.*, 2017; Eizaguirre-García *et al.*, 2000)." One panel member questioned the validity of relying on ecologic studies for causal inference or hazard identification. It is not clear why these studies are discussed as opposed to being triaged from consideration.

One panel member questioned the EPA's interpretation of the epidemiological studies with regard to developmental effects. The draft IRIS assessment in several statements concludes that there are some indications of an association between Cr(VI) exposure and developmental outcomes. For example, the draft IRIS assessment (p. 3-286) states that "In summary, there are some indications of an association between Cr(VI) exposure and spontaneous abortion, fetal growth, preterm birth, and congenital malformations, but the evidence is limited in quality and quantity." The draft IRIS assessment also states the following: "The evidence of an association between Cr(VI) exposure and developmental effects in humans is slight, with an indication of higher rates of spontaneous abortion with higher exposure levels in two of four low confidence paternal occupational exposure studies and an ecologic study with exposure evaluated at the zip code level (representing both maternal and paternal exposure). Results for other outcomes, including preterm birth, fetal growth, infant death, and congenital malformations indicated no clear association. The available evidence was all considered low confidence and the studies generally had poor sensitivity, so there is considerable uncertainty in this judgment." While both statements are quite cautious, one panel member suggested it may be more appropriate to interpret the epidemiological evidence as unsupportive of an association with developmental outcomes due to significant study limitations and inconsistent findings, rather than calling the evidence "slight" and "uncertain".

Mouse data were used to derive a developmental osRfD based on decreased F1 offspring postnatal growth utilizing the NOAEL of 11.6 mg/kg-day notes one panel member. Similar to the osRfD values for gastrointestinal effects in female mice or hepatic effects in female rats, EPA has not attempted to scientifically justify that the animal model utilized (mouse) is a better laboratory animal model than alternatives (e.g., rat) for the same or similar developmental effects in humans. However, contrary to that assumed for hepatic effects (female rats) and hematological effects (male rats), for developmental effects humans are assumed to have a dose-response (or POD at a minimum) similar to female mice (i.e., female mouse dose-response data, with dosimetric adjustments, are being used as the best laboratory animal surrogate data to represent dose-response in humans).

One panel member made the following statement, as noted previously in other sections of the charge question responses: It is not scientifically robust to simply state, "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and this statement could be equally applied to negative findings in a given laboratory animal species. When applicability of animal data to humans is not addressed scientifically, use of data

from a given animal species for dose-response assessment applied to humans can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species) that pertains directly to the meaningfulness of the resultant toxicity factor itself and for credibly informing risk/hazard management decisions. The limited uncertainty section (Section 4.1.6) does not address this larger uncertainty regarding the most appropriate animal model and whether a similar dose-response may or may not be expected in humans (after appropriate dosimetric adjustments). This is a major area of uncertainty, but it is not without precedent that it be explicitly acknowledged in the associated uncertainty section (e.g., EPA 2021). Furthermore, it can be a significant uncertainty that when considered amongst others can preclude a judgment that an osRfD meets the definition of an RfD (i.e., with uncertainty perhaps spanning an order of magnitude).

To provide additional support for a more in-depth consideration of species used for hazard identification, the panel member provided the following example. Zheng *et al.* (2018) is a high confidence study among the three studies reporting no effect on F1 growth (p. 3-293) and was rated high by EPA for growth endpoints (Table 3-46, p. 3-289). This rat study reported no change in pup body weight following maternal exposure at doses up to 12 mg/kg-d Cr(VI) via oral gavage from GD 12-21, which apparently did not demonstrate maternal toxicity, despite that Cr(VI) gavage exposure results in less effective gut detoxification (Section 4.1.1.3, p. 4-7). Thus, it does not appear that gavage exposure contributed to maternal toxicity or an unreasonably low POD for pup body weight based on Zheng *et al.* (2018), so perhaps it did not need to be excluded by EPA at least for this endpoint (p. 4-2, lines 10-14). This rat NOAEL (12 mg/kg-d) is very similar to the mouse POD utilized by EPA (11.6 mg/kg-d) and would enable the EPA to avoid any mouse maternal toxicity issues, potentially increasing confidence in the osRfD, in the event the mouse cannot be established as a better animal model for developmental effects in humans than the rat. In response to this comment by one panel member, another panel member noted that the issue raised here is irrelevant. Rather, whichever model provides the lowest dose is thereby,the most public health protective.

CQ3f: Hematological effects

One panel member noted the EPA should: (1) attempt to scientifically justify whether the mouse or rat is likely most biologically representative of humans such that the same or similar effects (gastrointestinal, etc.) are expected in humans at similar doses when converted to human equivalent doses (HEDs); and (2) in the event (1) cannot be established with sufficient scientific confidence, acknowledge within the assessment that the choice of the most appropriate laboratory animal model for prediction of Cr(VI)-induced adverse effects in humans has not been scientifically established (i.e., is not "settled science") but rather species selection is based on policy.

Charge Question #4

Below are detailed comments from one panel member.

Generally, the modeling approaches, model selection process, and benchmark response (BMR) levels used to derive PODs for toxicity value derivation are scientifically justified. However, as discussed previously, EPA should reconsider use of BMD results to derive the POD for diffuse epithelial hyperplasia of the duodenum in female mice (**Tier 2 suggestion**). Additionally, the following are comments on choice of dose metric for gastrointestinal effects (cancer and noncancer) and choice of model for gastrointestinal cancer effects.

Noncancer effects:

Use of BMD modeling to the extent possible, guided by standard statistical model fit criteria (+ visual inspection) for model selection, is essentially standard scientific procedure inside (and outside) EPA. As described in Section 4.1.2 for noncancer effects, EPA evaluated dose-response data the agency found amenable to BMD modeling with the models available in EPA's Benchmark Dose Software (BMDS, Version 3.2), estimating the BMD and the 95% lower confidence limit on the BMD (BMDL) using a BMR that represents a minimal, biologically significant level of change. The BMR levels selected (e.g., 10% extra risk, 1 SD) are justified and clearly described and presented for the effects that underwent BMD modeling in the respective subsections of Section 4.1.2. The estimated BMDLs were used as PODs and are summarized in Table 4-3 of the draft (pp. 4-12 to 4-13). Further details are included by EPA in Appendix D.1 (and EPA 2021). Section D.1.1 describes evaluation of model fit and model/BMDL selection for noncancer effects, which cites standard EPA guidance (i.e., EPA's Benchmark Dose Technical Guidance; EPA, 2012) on fitting models, comparing models, and calculating confidence limits to derive a BMDL for use as a POD. The steps outlined on p. D-7 for selecting a model for noncancer effects appear consistent with EPA BMD guidance.[3] Where BMD modeling for an effect was found by EPA not to be feasible, NOAELs or LOAELs were utilized as PODs and are summarized in Table 4-3.

As choice of dose metric is part of the modeling approach, a previous comment is reiterated here. Regarding choice of dose metric for BMD modeling of diffuse epithelial hyperplasia of the duodenum in female mice (NTP 2008), Haney (2015c) modeled this endpoint on the basis of duodenum tissue absorbed dose (mean added mg Cr/kg tissue at the same drinking water concentrations; Kirman *et al.* 2012), which is an available dose metric more closely causally associated with adverse effects (target tissue absorbed dose) and thus may generally be considered a more predictive and desirable (i.e., better) dose metric. The Log-Logistic and Dichotomous-Hill models provided adequate fit to the mouse data (Table 5 of Haney, 2015c) with a goodness-of-fit p value > 0.1 and scaled residuals <|2|, fit confirmed by visual inspection. These models provided almost identical fits (Figure 4 of Haney, 2015c) with the lowest AIC value, highest goodness-of-fit p value, the same BMD_{10} values and very similar $BMDL_{10}$ values (Table 6 of Haney, 2015c). The $BMDL_{10}$ values showed good agreement with their corresponding BMD_{10} values, all being within a factor of 1.5. Furthermore, the $BMDL_{10}$ values

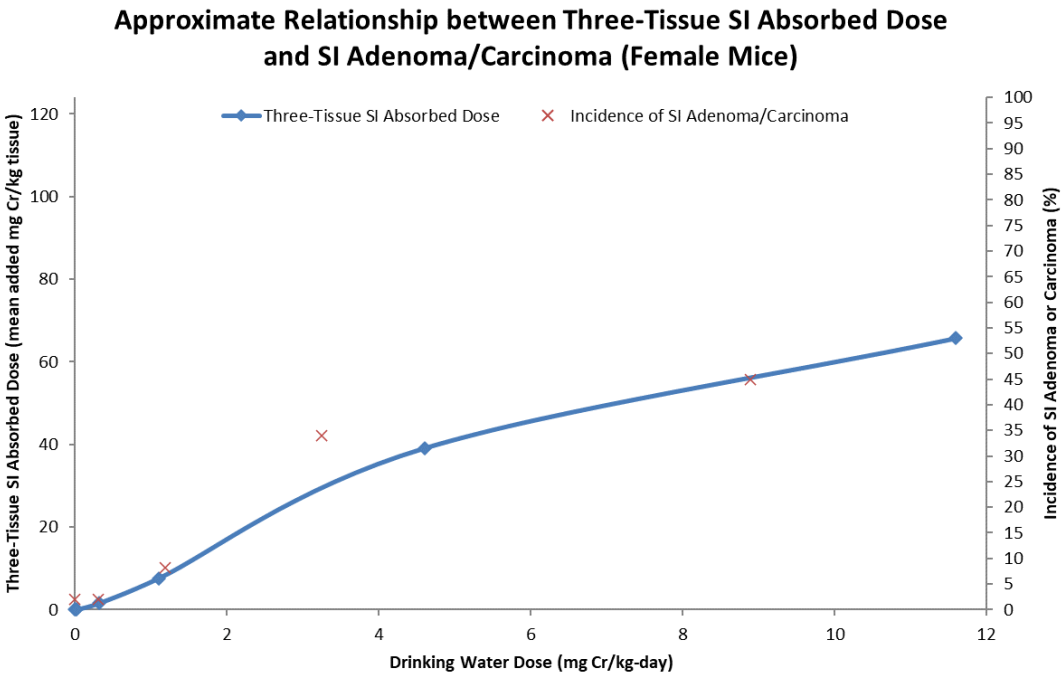
[3] Adequacy of model fit was judged on the basis of goodness-of-fit p-value ($p \geq 0.1$), scaled residuals (absolute value <2.0), and visual inspection of the model fit. Among all models providing adequate fit, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD (p. D-7).

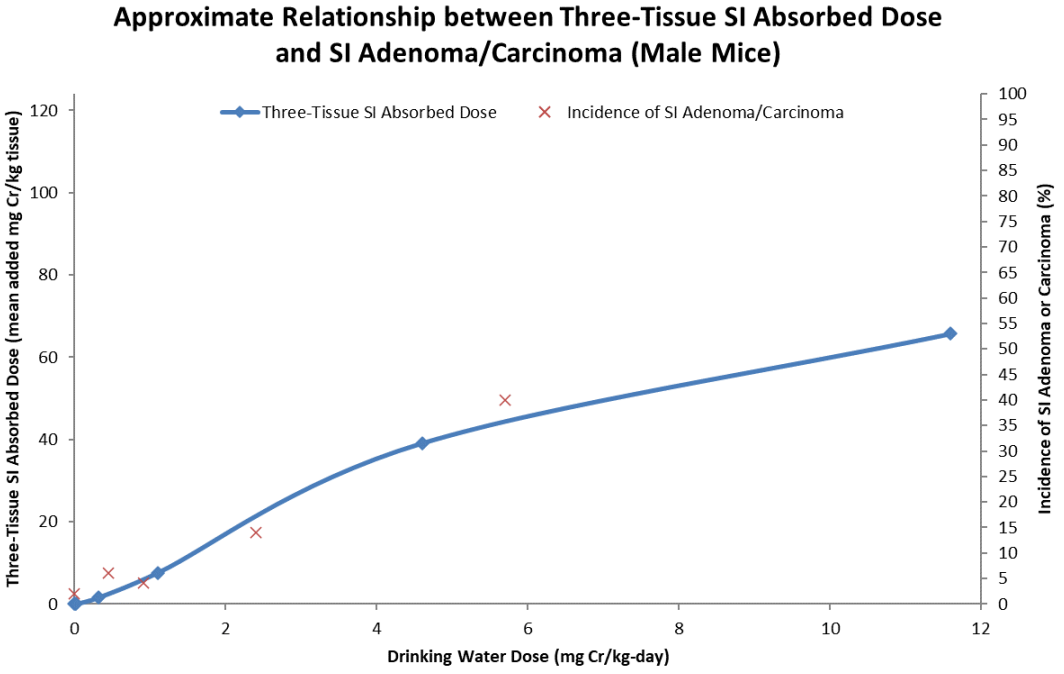
using mean added mg Cr/kg tissue as the internal target tissue dose metric were very similar to those using the 95% LCL of tissue concentrations (i.e., within a factor of 1.2), increasing confidence in use of these results. Unlike EPA BMD modeling results in Table D-5, the Log-Probit model had a $BMDL_{10}$ within a factor of 3 of the Log-Logistic and Dichotomous-Hill models, which had the lowest AIC. This BMD modeling approach: (1) did not exhibit the same problem as EPA's results (i.e., significantly different BMDs/BMDLs with BMDLs for the full dataset differing by as much as 27-fold; see p. 4-10 lines 13-16 and Table D-5); (2) meets all EPA modeling criteria in Section D.1.1 (on p. D-7); (3) is an approach that considers all the data and is generally considered more scientifically sophisticated than the NOAEL/LOAEL approach; and (4) is based on a dose metric more closely causally related to the toxic effect, target tissue absorbed dose. As a **Tier 2 suggestion**, one panel member suggests this BMD modeling approach be considered by EPA for use over their current problematic BMD modeling approach and scientifically outdated LOAEL approach. Accordingly, a similarly derived BMD/BMDL value based on EPA's own BMD modeling runs using this target tissue absorbed dose metric for diffuse hyperplasia in the duodenum of female mice could be used as the POD (e.g., a female mouse $BMDL_{10}$ of ≈1.39 added mg Cr/kg tissue) for the derivation of the RfD.

Cancer effects (gastrointestinal):

While similar comments are provided elsewhere below, choice of dose metric is of course part of the BMD modeling approach for gastrointestinal tumorigenesis/carcinogenesis and so comments of one panel member are also provided here. In regard to dose metric, target tissue absorbed dose is the most relevant and direct determinant of excess risk (e.g., all key events shown in Figure 3-16 of the draft occur following cellular uptake).[4] The figures below, provided in Appendix B with the underlying data, show the approximate relationship between mouse small intestine absorbed dose (mean added mg Cr/kg tissue) and the incidences of adenoma/carcinoma in the small intestine (SI) of female mice and male mice that EPA modeled (EPA 2021). There appears to be good agreement with the relevant data being from two mouse studies (i.e., Kirman *et al.,* 2012 mouse PBPK study and NTP 2008).

[4] The aim of cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks (EPA 2005a), and EPA (1992) indicates that for toxicological equivalence in cross-species scaling, equivalent target tissue concentrations of the carcinogenic moiety may be assumed to give rise to equivalent degrees of impact at the cellular level and yield equal cancer risks (Section II.B.3).





Based on the consideration of target tissue absorbed dose being the more direct determinant of excess risk, EPA may find that target tissue (e.g., three-tissue, duodenum, or duodenum + jejunum) absorbed dose provides a better fit to the data, at least in some cases. Target tissue absorption by the duodenum, jejunum, and ileum (or a subset) could be modeled to estimate the dose absorbed by each target tissue at the NTP (2008) study doses. A better fit to the

adenoma/carcinoma incidence data, despite the potential uncertainties in Table C-10 (p. C-18), would increase confidence in the use of target tissue absorbed dose (e.g., mean added mg Cr/kg tissue based on data from the Kirman *et al.* 2012 mouse PBPK study) as a preferred dose metric.

Nonlinearity observed in a dose-response curve often can be attributed to toxicokinetics (p. 3-5, EPA 2005a). As discussed later in these comments, target tissue absorption data (Kirman *et al.* 2012) indicate that the dose fractions absorbed by target tissues decrease as doses decrease below EPA's draft SFo (Oral Slope Factor; i.e., CSF) POD dose. For example, Figure 4 of Haney (2015a) shows dose fraction absorbed by target tissues (duodenum, jejunum, ileum) versus dose for drinking water concentrations of 0.3–60 mg SDD/L, which captures the draft SFo mouse POD dose and the dose at the MCL (Maximum Contaminant Level), and how the dose fraction absorbed at the draft SFo POD is higher than that at lower oral doses such as at the MCL.

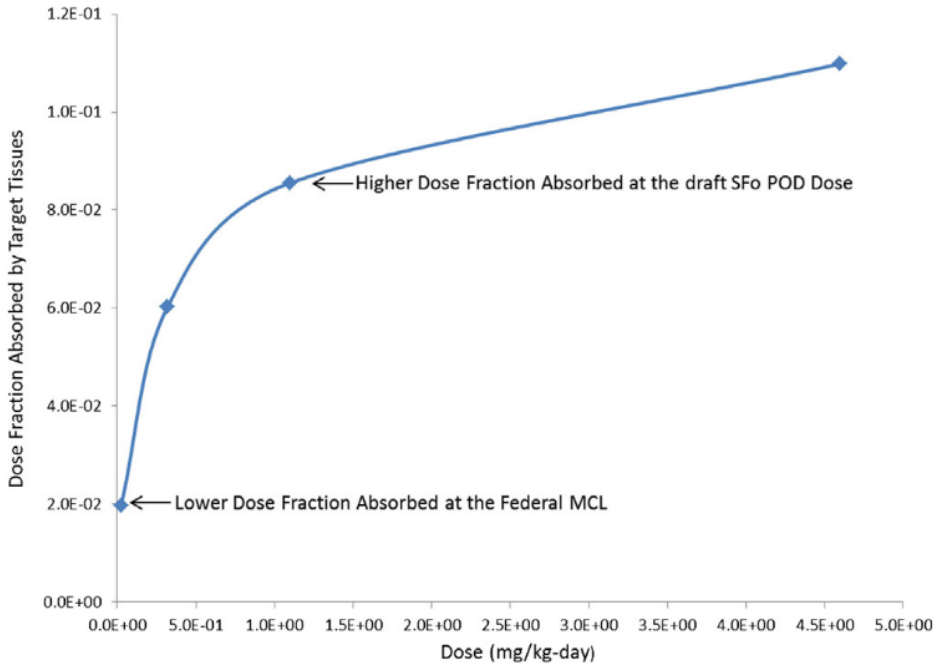
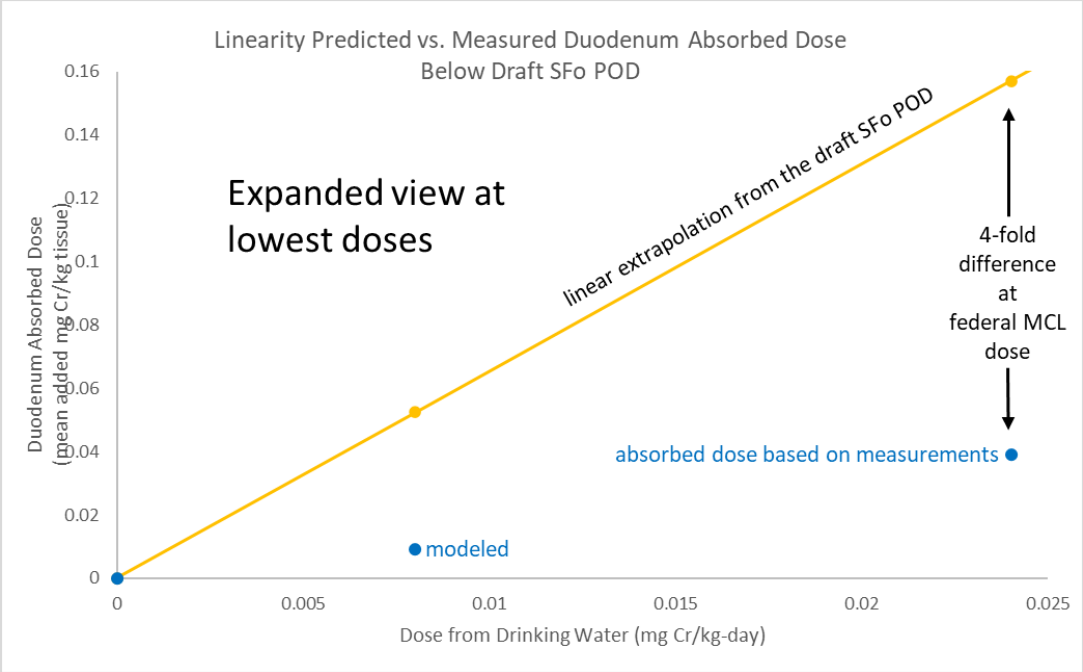
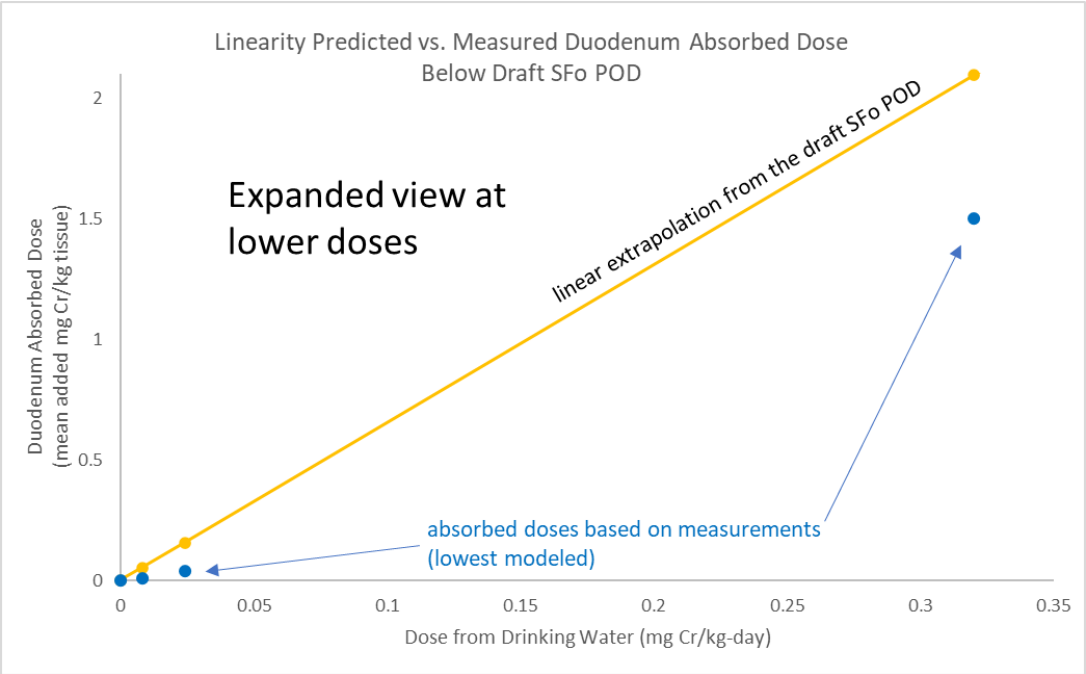


Fig. 4. Dose fraction absorbed versus dose.

Consequently, linear extrapolation of target tissue absorbed dose below the POD overpredicts target tissue absorbed dose. A duodenum-specific figure is provided below to help visualize this.



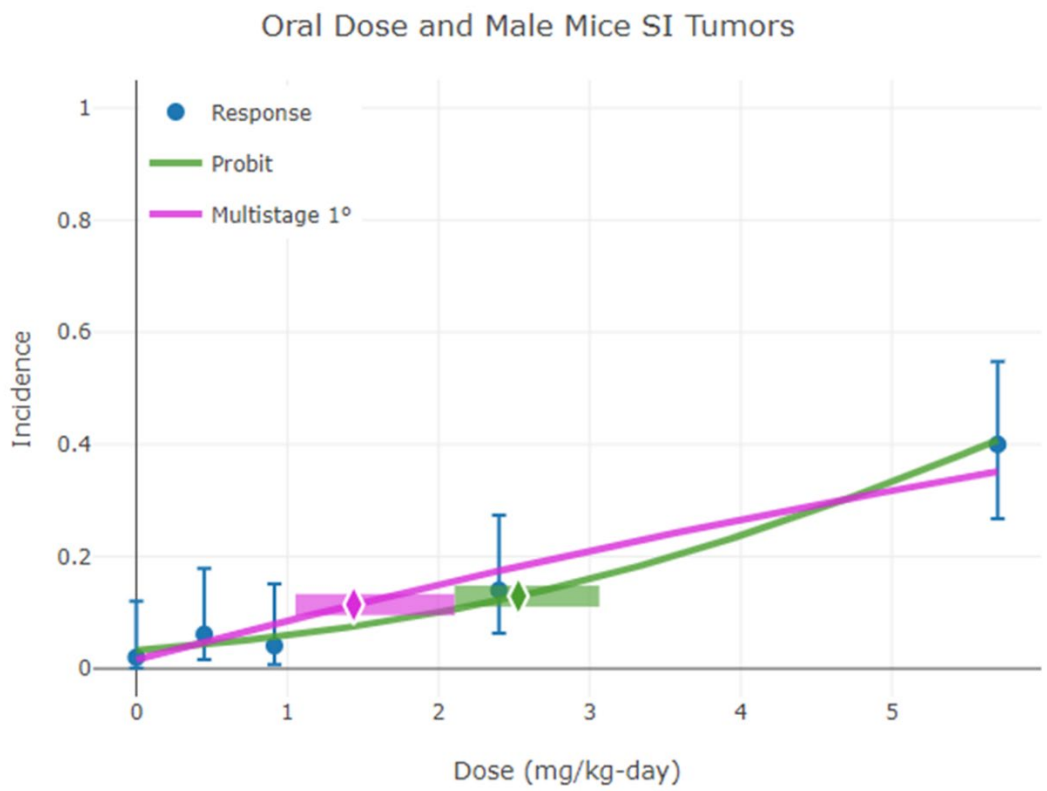
Importantly, consideration of target tissue Cr(VI) absorption by all three tissues (duodenum, jejunum, ileum) results in a similar overestimation at the MCL; a 4.3-fold overestimation (see Table 9 of Haney 2015a). Because target tissue absorption data indicate that the dose fractions absorbed by target tissues decrease as doses decrease below EPA's draft SFo POD, sublinearity should be expected at lower doses. An additional figure below from Appendix B also helps illustrate this.



Consistent with these comments one panel member recommends: (1) target tissue absorbed dose should be considered as a dose metric for modeling gastrointestinal cancer effects; and (2) models that appear sublinear over the lower part of the dose-response should also be considered for selection (e.g., if oral dose is used as the dose metric) as they may better reflect the sublinearity in dose-response expected based on the decreasing dose fractions absorbed by target tissues at low doses and also provide better fit to the data

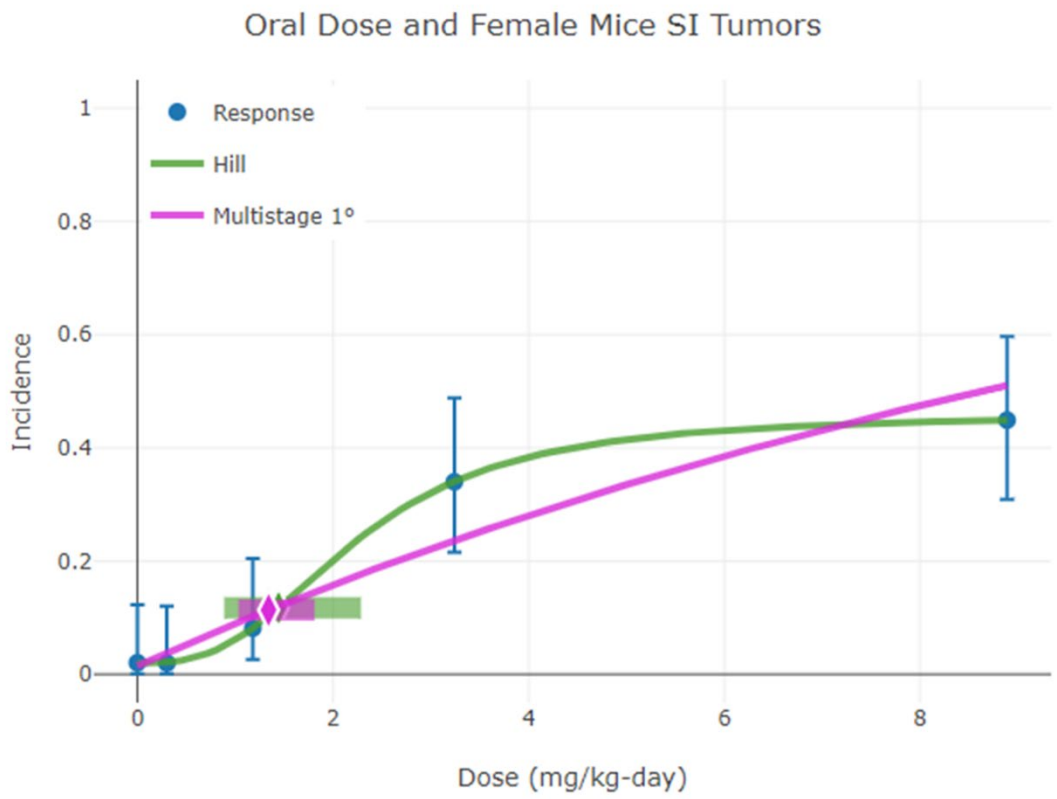
While EPA (2012) indicates that it is a current practice of the IRIS program to prefer the multistage model for cancer dose-response modeling of cancer bioassay data in the absence of data to support a more biologically based model, these guidelines also acknowledge that "dose-response modeling is largely a curve-fitting exercise among the variety of available empirical models" (pp. 26 and 57). Both the target tissue absorption data and the lack of scientific consensus on the carcinogenic MOA (see comments under question 6a) suggest that other models should be considered. Better fit by models other than that selected by EPA (multistage degree 1) reflect a better fit to the reality of the actual dose-response data and the underlying biology that produced those results. Better fitting models might also appear somewhat sublinear across lower oral doses, more accurately reflecting the actual dose-response data and perhaps also being more consistent with the expectation of low-dose sublinearity (e.g., see the figures above; Appendix B; Haney 2015a,b,c). It is further noted that while guidance can provide a good general framework for decision-making and/or promoting consistency (e.g., EPA 2012, 2014), decisions such as (but not limited to) model selection in a particular case are best made (i.e., most defensible) when based on what is best supported scientifically in that specific case (i.e., best available science as evaluated on a case-by-case basis). Accordingly, citing general modeling guidance as justification for selection of a poorer-fitting default model over a model that better fits the dose-response data in a specific case should not be considered a scientific defense (i.e., policy may not result in best available science in a particular case, and general guidance and defaults should not be considered to scientifically outweigh statistical model fit criteria and visual confirmation of better fit to the actual dose-response data that the selected model should most accurately represent). Moreover, the EPA cancer guidelines (EPA 2005a) allow for different model-fitting approaches for tumor data when justification is provided (comparisons should also be made to results from standard EPA procedures; see p. 1-10 of EPA 2005a). One panel member recommends that this should be considered.

Consistent with the comments above some exploratory BMD analyses were conducted using EPA's BMDS Online. First, relevant to "(2)" above, the same oral dose and small intestine (SI) tumor data that EPA modeled for male and female mice (pp. 87 and 91 of EPA 2021) were simply re-run to determine if EPA's software suggests a different model than EPA selected (multistage degree 1) based on better fit. For male mice, EPA's software suggested use of the probit model over other models including that selected by EPA (multistage degree 1). The probit model had a lower AIC (161.933) than EPA's selected model (163.261) and fit the data much better visually because the data themselves do not appear linear across the dose range.



Additionally, its sublinear shape below the BMD_{10}/$BMDL_{10}$ is more consistent with the sub-linearity expected across low oral doses based on target tissue absorption data (discussed above). Nonlinearity observed in a dose-response curve often can be attributed to toxicokinetics (p. 3-5, EPA 2005a). The one panel member noted that there was no explicit discussion in the draft IRIS assessment as to why such a model should not be selected, but such a discussion should be included for transparency if EPA retains oral dose as the dose metric for BMD modeling (**Tier 2 suggestion**). See Appendix to CQ#4 comments for a summary of these BMD results.

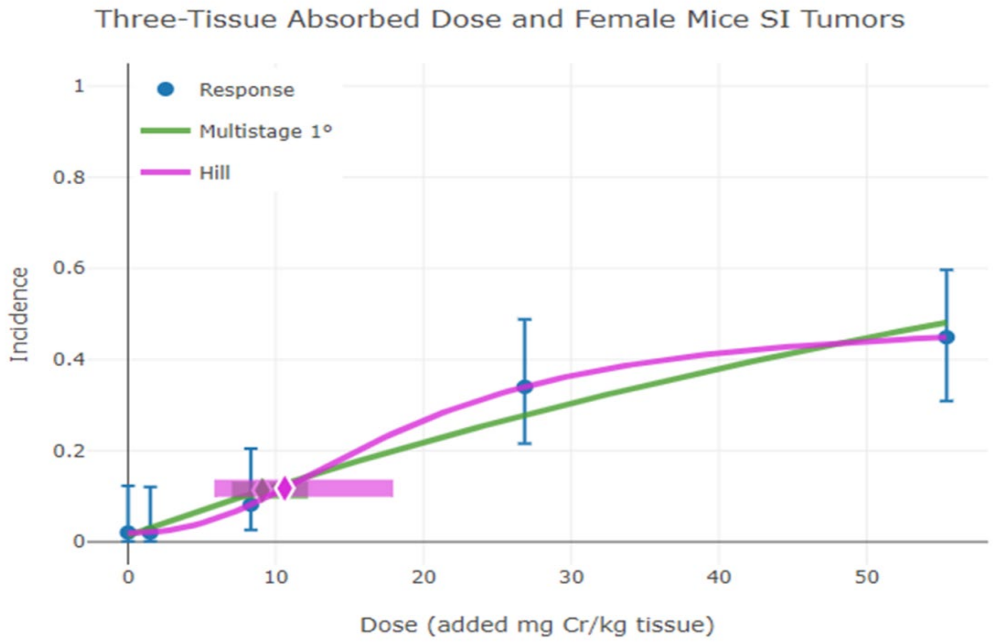
For female mice, EPA's software suggested use of the Hill model over other models including that selected by EPA (multi-stage degree 1). The Hill model had a lower AIC (186.802) than EPA's selected model (187.131), and since the dose-response data suggest a plateau, it is reasonable to fit the Hill model (EPA 2012). All scaled residuals are less than an absolute value of 0.053. Visually, the Hill model fits the dose-response data much better than EPA's selected model because the data themselves do not appear linear across the oral dose range.



Additionally, its sublinear shape below the BMD_{10}/$BMDL_{10}$ is more consistent with the sub-linearity expected across low oral doses based on target tissue absorption data (discussed above). Nonlinearity observed in a dose-response curve often can be attributed to toxicokinetics (p. 3-5, EPA 2005a). One panel member again noted there was no explicit discussion in the draft IRIS assessment as to why such a model should not be selected, but such a discussion should be included, according to this panel member for transparency if EPA retains oral dose as the dose metric for BMD modeling.

In the view of one panel member, relevant to "(1)" above, as target tissue absorbed dose should be a preferred dose metric, target tissue absorbed dose (added mg Cr/kg tissue) was estimated at the same doses (mg/kg-d) that EPA used to model gastrointestinal tumors in male and female mice (pp. 87 and 91 of EPA 2021). Not being a PBPK modeler, one panel member for purposes of providing examples, the approximations were based on the target tissue absorption data (Table 2 of Haney 2015a based on Kirman *et al.* 2012) simply by interpolating the target tissue absorbed dose for the dose of interest based on the slope of the line between the two target tissue absorption data points that bracketed the dose of interest (see Appendix to CQ#4 -BMDS results). EPA PBPK modelers could provide better estimates (e.g., by fitting a line/function through the target tissue absorption data). Then, these estimates of target tissue absorbed dose (at the same doses EPA used for modeling) were used for BMD modeling of male and female mouse SI tumor incidence (Appendix to CQ#4 -BMDS results). However, as the target tissue absorption data were collected in female mice, the example provided below is specific to female mice.

Using the target tissue absorbed-dose estimates for female mice, EPA's software suggested use of the same model selected by EPA (multistage degree 1). However, by visual inspection the Hill model may be interpreted to fit better (albeit with a somewhat higher AIC; see the figure below). Regardless, *an important observation is that when target tissue absorbed dose is used as the dose metric for tumors in female mice, EPA's BMDS changes from recommending the Hill model, which is sublinear at low oral doses (see the figure above), to EPA's draft selected linear model* (multistage degree 1). Thus, it appears that use of target tissue absorbed dose as the dose metric tends to linearize the tumor dose-response in female mice, the sex for which the target tissue data were collected. *That is, not only does EPA BMDS change to suggest a linear model when target tissue absorbed dose is used as the dose metric, but the fit for the Hill model* (shown on both figures and suggested by EPA BMDS when oral dose is used as the dose metric) *appears appreciably flatter compared to using oral dose*.



While using crude target tissue absorbed dose estimates did not result in EPA's BMDS recommending a linear model for SI tumors in male mice, use of more scientifically sophisticated estimates derived by EPA PBPK modelers may result in EPA's BMDS recommending a linear model for male mouse tumors or at least make the recommended model appear more linear in the view of one panel member.

Importantly, it is noted that when target tissue absorbed dose is used as the dose metric for female mouse tumors, EPA's software suggested/draft assessment selected model (multistage degree 1) has an appreciably lower AIC (184.577) than the same model with the draft assessment dose metric (in mg/kg-d; 187.131). In fact, using target tissue absorbed dose as the dose metric, the majority of the BMD models have lower AIC values than the AIC for the model selected by EPA (187.131) using the draft assessment dose metric (mg/kg-d; see summary results in Appendix to CQ#4 -BMDS results). This appears to suggest that target tissue absorbed dose is a better dose metric not only because it is more proximally/causally related to excess risk, and

consistent with this, it also appears to result in better model to fit the resultant tumor data in the view of one panel member.

These exploratory BMD results help demonstrate a recommendation from this one panel member that is a **Tier 1 necessary revision**: (1) target tissue absorbed dose should be considered by EPA as a dose metric for modeling gastrointestinal cancer effects; and (2) models that appear sublinear over the lower part of the dose-response should be considered for selection by EPA (e.g., if oral dose is used as the dose metric) as they may provide a better fit to the tumor dose-response data than the model selected by EPA (multistage degree 1) and also may better reflect the sublinearity in dose-response expected based on the decreasing dose fractions absorbed by target tissues across lower doses (discussed above and elsewhere). All this being said, given that the dose absorbed by target tissues is the more proximate causal determinant of toxicity such as carcinogenic excess risk (e.g., all key events shown in Figure 3-16 of the draft IRIS assessment occur following cellular uptake),[5] and given all that is known about Cr(VI) toxicokinetics and that EPA has PBPK modelers, one panel member suggests that EPA should attempt utilizing target tissue absorbed dose as a dose metric and exploring the implications of nonlinearities in Cr(VI) toxicokinetics for selecting the most appropriate dose-response model or low dose extrapolation method. These are reasonable considerations for EPA to explore in order to produce a sufficiently scientifically diligent and rigorous assessment, even in the absence of the information and analyses contained within these comments.

[5] The aim of cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks (EPA 2005a), and EPA (1992) indicates that for toxicological equivalence in cross-species scaling, equivalent target tissue concentrations of the carcinogenic moiety may be assumed to give rise to equivalent degrees of impact at the cellular level and yield equal cancer risks (Section II.B.3).

APPENDIX- BMDS MODELING RESULTS

Oral Dose vs. Male Mice SI Tumors

Report generated: 2023-Jan-22 23:01 UTC
Analysis URL: View / Update
BMDS version: 3.3.0 (2022.10)
BMDS online version: c22f10c1 (2022-10-06)

Session Results

Input Dataset

Dose (mg/kg-day)	N	Incidence
0	50	1
0.45	49	3
0.914	49	2
2.4	50	7
5.7	50	20

Input Settings

Setting	Value
BMR	10% Extra Risk
Confidence Level	0.95
Maximum Multistage Degree	3

Frequentist Summary

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	Recommendation and Notes
Hill	1.128	2.238	3.571	0.588	163.929	-0.011	-0.48	**Viable**
Gamma	1.146	2.218	3.525	0.585	163.936	-0.025	-0.465	**Viable**
LogLogistic	1.128	2.238	3.571	0.588	163.929	-0.011	-0.48	**Viable**
Multistage 1°	1.053	1.439	2.106	0.607	163.261	-0.961	0.232	**Viable**
Multistage 2°	1.162	2.155	3.197	0.645	163.76	0.016	-0.271	**Viable**
Multistage 3°	1.165	2.178	3.583	0.367	165.732	0.11	-0.19	**Viable**
Weibull	1.15	2.197	3.622	0.599	163.889	-0.013	-0.409	**Viable**
Logistic	2.295	2.741	3.274	0.76	162.191	0.546	-0.606	**Viable**
LogProbit	1.154	2.336	3.458	0.57	164.03	0.028	-0.63	**Viable**
Probit[ab]	2.107	2.529	3.064	0.807	161.933	0.368	-0.493	**Recommended - Lowest AIC**
Quantal Linear	1.053	1.439	2.106	0.607	163.261	-0.961	0.232	**Viable**

[a] Recommended best-fitting model
[b]

Oral Dose vs. Female Mice SI Tumors

Report generated: 2023-Jan-22 23:01 UTC
Analysis URL: View / Update
BMDS version: 3.3.0 (2022.10)
BMDS online version: c22f10c1 (2022-10-06)

Session Results

Input Dataset

Dose (mg/kg-day)	N	Incidence
0	49	1
0.302	50	1
1.18	49	4
3.24	50	17
8.89	49	22

Input Settings

Setting	Value
BMR	10% Extra Risk
Confidence Level	0.95
Maximum Multistage Degree	3

Frequentist Summary

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	Recommendation and Notes
Hill[a]	0.888	1.444	2.286	0.943	186.802	0.007	0.048	**Recommended - Lowest AIC**
Gamma	1.034	1.341	2.098	0.34	187.131	-0.451	0.308	**Viable**
LogLogistic	0.801	1.228	2.013	0.274	188.156	-0.574	0.388	**Viable**
Multistage 1°	1.034	1.341	1.812	0.34	187.131	-0.451	0.308	**Viable**
Multistage 2°	1.034	1.341	1.932	0.34	187.131	-0.451	0.308	**Viable**
Multistage 3°	1.034	1.341	1.932	0.34	187.131	-0.451	0.308	**Viable**
Weibull	1.034	1.341	2.059	0.34	187.131	-0.451	0.308	**Viable**
Logistic	2.677	3.222	3.903	0.003	198.472	3.103	-1.296	**Questionable** Goodness of fit p-value less than 0.1 Abs(Residual of interest) greater than 2.0
LogProbit	0.693	1.247	2.005	0.38	187.353	-0.554	0.304	**Viable**
Probit	2.471	2.963	3.599	0.006	196.932	2.985	-1.158	**Questionable** Goodness of fit p-value less than 0.1 Abs(Residual of interest) greater than 2.0
Quantal Linear	1.034	1.341	1.812	0.34	187.131	-0.451	0.308	**Viable**

[a] Recommended best-fitting model

Drinking Water Dose (mg Cr/kg-day)	Three-Tissue SI Absorbed Dose (mean added mg Cr/kg tissue)	Slope from Next Lower Dose (mean added mg Cr/kg tissue per mg Cr/kg-day)	Male Mouse Dose (mg Cr/kg-day)	Male Mouse Three-Tissue Dose (approximate mean added mg Cr/kg tissue)	N	Incidence	Female Mouse Dose (mg Cr/kg-day)	Female Mouse Three-Tissue Dose (approximate mean added mg Cr/kg tissue)	N	Incidence
0	0		0	0	50	1	0	0	49	1
0.024	0.039	1.625	0.45	2.589166667	49	3	0.302	1.494743243	50	1
0.32	1.589	5.236486486	0.914	6.158992308	49	2	1.18	8.310228571	49	4
1.1	7.59	7.693589744	2.4	19.29371429	50	7	3.24	26.85611429	50	17
4.6	39.1	9.002857143	5.7	43.28	50	20	8.89	55.402	49	22

Absorbed dose data from Table 2 of Haney (2015a) based on Kirman et al. (2012).

Three-Tissue Absorbed Dose vs. Male Mice SI Tumors

Report generated: 2023-Jan-22 19:01 UTC
Analysis URL: View / Update
BMDS version: 3.3.0 (2022.10)
BMDS online version: c22f10c1 (2022-10-06)

Session Results

Input Dataset

Dose (added mg Cr/kg tissue)	N	Incidence
0	50	1
2.589	49	3
6.159	49	2
19.294	50	7
43.28	50	20

Input Settings

Setting	Value
BMR	10% Extra Risk
Confidence Level	0.95
Maximum Multistage Degree	3

Frequentist Summary

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	Recommendation and Notes
Hill	8.629	18.461	28.113	0.58	163.984	-0.006	-0.597	**Viable**
Gamma	8.825	18.453	27.718	0.577	163.994	-0.008	-0.602	**Viable**
LogLogistic	8.629	18.461	28.113	0.58	163.984	-0.006	-0.597	**Viable**
Multistage 1°	8.029	11.045	16.282	0.566	163.424	-0.86	0.091	**Viable**
Multistage 2°	8.933	17.453	24.669	0.609	163.852	-0.053	-0.422	**Viable**
Multistage 3°	8.989	17.581	27.9	0.637	163.782	0.057	-0.312	**Viable**
Weibull	8.847	18.21	28.539	0.581	163.964	-0.013	-0.553	**Viable**
Logistic	17.478	20.87	24.927	0.786	162.047	0.386	-0.606	**Viable**
LogProbit	9.216	18.9	27.129	0.583	164.022	0.013	-0.683	**Viable**
Probit[ab]	16.043	19.263	23.353	0.812	161.872	0.198	-0.505	**Recommended - Lowest AIC**
Quantal Linear	8.029	11.045	16.282	0.566	163.424	-0.86	0.091	**Viable**

[a] Recommended best-fitting model
[b]

Three-Tissue Absorbed Dose vs. Female Mice SI Tumors

Report generated: 2023-Jan-22 19:01 UTC
Analysis URL: View / Update
BMDS version: 3.3.0 (2022.10)
BMDS online version: c22f10c1 (2022-10-06)

Session Results

Input Dataset

Dose (added mg Cr/kg tissue)	N	Incidence
0	49	1
1.495	50	1
8.31	49	4
26.856	50	17
55.402	49	22

Input Settings

Setting	Value
BMR	10% Extra Risk
Confidence Level	0.95
Maximum Multistage Degree	3

Frequentist Summary

Model	BMDL	BMD	BMDU	*P*-Value	AIC	Scaled Residual for Dose Group near BMD	Scaled Residual for Control Dose Group	Recommendation and Notes
Hill	5.841	10.607	17.95	0.948	186.801	0.006	0.044	**Viable**
Gamma	7.041	9.772	16.402	0.524	186.528	-0.383	0.292	**Viable**
LogLogistic	5.738	9.716	16.13	0.627	186.058	-0.376	0.273	**Viable**
Multistage 1°[ab]	7.026	9.09	12.189	0.71	184.577	-0.501	0.358	**Recommended - Lowest AIC**
Multistage 2°	7.026	9.09	15.199	0.71	184.577	-0.501	0.358	**Viable**
Multistage 3°	7.026	9.09	15.199	0.71	184.577	-0.501	0.358	**Viable**
Weibull	7.032	9.509	16.103	0.513	186.558	-0.429	0.319	**Viable**
Logistic	17.525	20.897	24.876	0.04	193.008	2.333	-1.046	**Questionable** Goodness of fit p-value less than 0.1 Abs(Residual of interest) greater than 2.0
LogProbit	5.096	9.818	15.988	0.735	185.659	-0.349	0.152	**Viable**
Probit	16.215	19.288	23.002	0.074	191.358	2.154	-0.877	**Questionable** Goodness of fit p-value less than 0.1 Abs(Residual of interest) greater than 2.0
Quantal Linear	7.026	9.09	12.189	0.71	184.577	-0.501	0.358	**Viable**

[a] Recommended best-fitting model
[b]

Charge Question #5

One panel member commented that the use of the interspecies uncertainty factor of 3 for nasal pathology is appropriate, but not for bronchoalveolar/lung pathology: rodents contain 10-20x more ascorbate in the bronchoalveolar fluid than humans, which offers a similarly higher magnitude of protection against cytotoxic and genotoxic effects of soluble Cr(VI) due to its extracellular reduction/detoxification (PMID: 28759204, Krawic C. *et al.*. 2017 and refs. therein).

Charge Question #6a

A large majority of the panel (12 of 14 members) agreed that the evidence that Cr(VI) causes cancer through a mutagenic mode of action was sufficiently supported in experimental systems and was relevant to humans. In the panel's discussions several panel members noted that public comments, indicating that the EPA should not use the same criteria for evaluating genotoxicity studies for hazard identification as it uses for mechanistic studies, have merit (e.g., see cited comments by Dr. Toby Rossman and Dr. Sam Cohen in Appendix B, Charge Question - 6a4 below). Genotoxicity studies designed to evaluate the MOA for tumors observed in carcinogenicity bioassays, such as the NTP (2008) chronic oral study of hexavalent chromium, should not require dose levels that reach the maximum tolerated dose (MTD) if tumors were observed below the MTD. In contrast, studies intended to identify whether a previously untested agent is genotoxic should include a wider range of doses (i.e., including the MTD or similar type of dose).

A small portion of the panel (2/14) stated that there was insufficient evidence to support the mutagenic MOA for carcinogenesis conclusion, particularly given the lack of genotoxic/mutagenic activity recently reported in the mouse GI tract. This small portion of the panel found that there was substantial evidence in support of a cytotoxicity/regenerative hyperplasia MOA. One of these members wrote that "*EPA has demonstrated genotoxicity hazard but has not conducted a MOA analysis that demonstrates a mutagenic MOA for carcinogenicity. Moreover, EPA's MOA conclusion is far from representing scientific consensus (i.e., "settled science") as scientists at other notable agencies and elsewhere have concluded that an alternate MOA is best supported by the weight of available relevant scientific evidence."* A panel member indicated that the evidence for both routes but, particularly through the oral route, was insufficient to conclude that Cr(VI) induces cancer through a mutagenic MOA. The one panel member noted that the conclusions of other regulatory authorities such as Canada, Japan, Texas and the WHO (see Health Canada 2016, FSCJ 2019, TCEQ 2016, WHO 2020), stated that the evidence more strongly supported a cytotoxicity/regenerative hyperplasia MOA and that this should be explicitly acknowledged in the draft IRIS assessment. This one panel member had extensive additional comments on statements in the assessment which can be found here, in Appendix B, Charge Question - 6a4 below.

The second panel member who had a minority opinion recommended that the EPA should conduct a parallel evaluation of the two hypothesized modes of action (mutagenic and cytotoxicity/regenerative hyperplasia, informed by the International Programme for Chemical

Safety (IPCS) MOA framework, as well as adverse outcome pathway (AOP) considerations. The MOA framework is embodied in the 2006 IPCS framework, with elements also incorporated into the EPA Cancer Risk Assessment Guidelines (EPA, 2005a) and has been expanded upon over time by an international group of scientists that had representation from EPA (e.g., Boobis *et al.*, 2006). The framework involves proposing a plausible MOA using a series of key events and evaluating the weight-of-evidence of the key events, as informed by the Bradford Hill postulates (e.g., dose-response, temporality). If multiple MOAs are under consideration (as is the circumstance here), individual MOAs need their own framework analysis. Several citations for the MOA framework, as well as AOP considerations can be found in the responses to Charge Question 2 in Appendix B (e.g., Sonich-Mullin *et al.*, 2001, Boobis *et al.*, 2006, Becker *et al.*, 2017). It was also noted that, as part of their MOA evaluation, both Bhat et al (2020) and Haney (2015) conducted a comparative analysis of a mutagenic and a nonmutagenic MOA. In discussing this topic, the majority of the panel noted that Section D.3.3. of the draft IRIS Supplemental Information document already includes an evaluation of the alternate threshold MOA that presents a RfD of 9 $x10^{-4}$ mg/kg-day for diffuse epithelial hyperplasia of the mouse small intestine.

The panel also noted that many of the EPA's conclusions were based on experiments where the doses and exposure concentrations were much higher than would be experienced by the general population. The majority of the panel (13 of 14) indicated that additional examination is warranted in evaluating the carcinogenic risks at low to ultra-low doses. Since key steps or a combination of steps in the transformation, uptake, and detoxication of Cr(VI) and its derived reactive species could result in deviations from linearity in response, the panel recommended that the EPA re-examine its conclusions to ensure that they are consistent with modeled or expected results. More discussion of these toxicokinetic factors and their possible low dose implications can be found in Appendix B, Charge Question – 6a3.

As noted above the large majority of the panel (12 of 14 members) agreed that the evidence that Cr(VI) causes cancer through a mutagenic mode of action was sufficiently supported. One panel member had several recommendations for revisions of the MOA sections as enumerated below to encourage EPA to strengthen their analyses and substantiate scientific basis for the assessment conclusions **(**This one panel member stated that the following should be a Tier 1 recommendation: Necessary Revision). These detailed comments are provided below.

Overall Comments Mutagenic Mode of Action (MOA) from one panel member:
Cr(VI)-containing compounds have been found genotoxic in a large variety of cells and organisms, ranging from bacterial, yeast, flies to vertebrates and humans. Cr(VI) was also consistently mutagenic in standard bacterial (Ames assay) and mammalian (*Hprt*-targeted mutagenesis) test systems. Mutagenicity and genotoxicity of Cr(VI) in various biological systems (De Flora 1990 PMID: 2407950; McCaroll *et al.* 2010 PMID: 19708067) and specific forms of Cr-DNA damage have all been reviewed in details (Zhitkovich 2011, Krawic and Zhitkovich, 2023). Studies from JP Wise laboratory established the ability of Cr(VI) to cause chromosomal damage in cells from many nontraditional vertebrate animals.

<u>The most serious omission</u> in the presentation of the mutagenic MOA in the draft IRIS assessment is a near complete absence of information on

mutagenicity/genotoxicity/clastogenicity of Cr(VI) in standard *in vitro* test systems and in many model organisms (other than laboratory rodents). This omission makes the case for the mutagenic MOA appear somewhat weaker than it really is, as the draft IRIS assessment relies too heavily on a more limited set of studies in rodents and humans. Importantly, standard mutagenicity test systems (Ames assay, mammalian micronucleus and mutagenesis assays alone and especially, in combination) have a high predictive power for carcinogenicity in rodents (original Refs are cited in Zhitkovich 2011), making a stronger case for the mutagenic MOA in Cr(VI) carcinogenicity.

The mutagenicity and genotoxicity of Cr(VI) result from a direct DNA-damaging mechanism, as evidenced by the formation of mutagenic chromium-DNA adducts and other genotoxic DNA damage (chromosomal breaks, DNA-protein crosslinks). Formation of chromium-specific DNA lesions at environmentally relevant Cr(VI) concentrations and sensitivity of genotoxic responses to manipulations of cellular DNA repair further support the role of direct DNA damage as a primary cause of genotoxicity. Since chromate [the solubilized form of Cr(VI)] is taken up via ubiquitously expressed sulfate transporters and a reductive activation of Cr(VI) in cells occur nonenzymatically via direct chemical reactions with ubiquitous ascorbate, glutathione and cysteine, there is no reason to believe that the formation of DNA damage in the intestinal cells and in more extensively studied cell types (fibroblasts, for example) would be different. In fact, both human colon (Peterson-Roth E. *et al.* 2005) and human lung cells (Reynolds M. *et al.* 2004; 2007) showed a linear dose-dependent formation of Cr-DNA adducts and DNA double-strand breaks. Thus, diverse lines of evidence are fully consistent with a mutagenic mode of carcinogenic action for Cr(VI). The draft IRIS assessment clearly presented the main arguments for this designation from rodent and human studies, carefully considering the limitations of findings based on specificity and sensitivity. However, the conclusion on the MOA needs to be strengthened by the inclusion of available information on Cr(VI) mutagenicity and genotoxicity in many model systems (*in vitro* or nonrodent species) and direct mechanisms of Cr(VI) mutagenicity and genotoxicity via the formation of Cr-specific forms of DNA damage (adducts, DNA-protein crosslinks). A section on KC#1 DNA Reactivity (p. 3-122) should be expanded to provide evidence for the generation of mutagenic DNA damage in defined reduction systems (with ascorbate, glutathione, cysteine) to further support a direct DNA-reactivity mechanism for Cr(VI) via its metabolites. This section should be directly discussed/linked in the presentation of the mutagenic MOA. In the absence of the direct genotoxic mechanism, even the mutagenic MOA could be highly nonlinear if it results from indirect effects (altered metabolism by high doses, for example).

One panel member also agreed with the EPA's overall conclusion on findings in human studies, which due to their correlative nature and imprecise assessment of exposures and co-exposures can be best evaluated as a group. Specifically, EPA concluded that findings on chromosomal damage in human studies are largely consistent with the association of Cr(VI) exposure and chromosomal abnormalities across a range of exposure types and geographic locations. Although these observations were only available from studies rated as low confidence and a single medium confidence, a large evidence base diminishes concerns about deficiencies in any single low confidence study. Analysis of DNA-protein crosslink measurements in human populations (as detailed below) should strengthen the evidence based in human studies.

1) *P. 3-92 (lines 10-13): "Studies measuring DNA damage or indicators of DNA damage or using more direct methods of chemical administration (i.e., i.p. injection) were not prioritized but are still considered as supplemental evidence to mutation and are summarized in Appendix C.3.2.2.*"

One panel member did not understand why the EPA decided to deemphasize DNA damage measurements. The panel member was referring to the more specific forms of Cr-DNA damage (Cr-DNA adducts, DNA-protein crosslinks) rather than oxidative damage. The draft IRIS assessment contains extensive information on measurements of 8-oxodG and chromosomal damage (including micronuclei) which may in some circumstances result from indirect genotoxic mechanisms. Cr-DNA adducts and DNA-protein crosslinks are more direct markers of DNA damage by Cr(VI). Cr-DNA adducts were abundantly present in Cr(VI)-treated animals at doses that did not induce 8-oxodG which was measured by a specific HPLC method (Yuann JM 1999, PMID: 10383900).

Additional information on DNA-protein crosslinks (related to comment #3 below) from one panel member:

Formation of DNA-protein crosslinks (DPC) in cultured cells has been established by many studies over more than 30 years. Importantly, these DNA lesions were also detected in Cr(VI)-treated rodents and other animals, demonstrating genotoxicity of Cr(VI) *in vivo*.

<u>DPC studies in rodents</u>:

Coogan TP (1991, PMID: 2038750): using Fisher 344 rats, found increased DPC in liver following 3 weeks of exposure at both 100 and 200 ppm Cr(VI) [K_2CrO_4] in drinking water and i.p. dosing with Cr(VI).

Izzotti A. (1998, PMID: 9685658): detected DPC formation in the lung after intratracheal instillation of Na-chromate in rats.

Tsapakos MJ (1981, PMID: 7217049; 1983, PMID: 6640521): found higher levels of DPC in liver, kidney and lung of chromate-exposed rats.

Zhitkovich and Costa (1992, PMID: 1499101): found increased levels of DPC in white blood cells of mice and rats exposed to Cr(VI) by i.p.

<u>Human studies</u>:

Elevated levels of DPCs have been found in chromium-exposed human populations (early studies reviewed in Zhitkovich *et al.* 1998, PMID: 9703480).

Genotoxic significance of DPCs:

DPCs are now firmly established as replication stress-inducing DNA lesions triggering activation of the DNA damage-responsive kinase ATR (Wong VC 2012, PMID: 22722496; Duxin JP 2014, PMID: 25303529).

Replication stress is recognized as a major contributor to the formation of genomic rearrangements/mutations and genomic instability in cancer:

Gaillard, H., García-Muse, T. & Aguilera, A. Replication stress and cancer. *Nat Rev Cancer* **15**, 276–289 (2015).

Macheret M, Halazonetis TD. DNA replication stress as a hallmark of cancer. *Annu Rev Pathol*. 2015: 10:425-448.

DPCs are tumorigenic, as evidenced by the tumor formation in mice defective in the DPC-removing protease SPRTN (PMID: 30170832, Fielden J. 2018 – for review)

Mechanistic information on micronuclei formation by Cr(VI) from one panel member:

It is important to add that the majority of micronuclei induced by Cr(VI) in human cells with physiological levels of ascorbate were clastogenic (centromere-negative) and their main source was a direct Cr-DNA damage (Cr-DNA adducts), evidenced by the sensitivity of micronuclei formation to manipulations of DNA mismatch repair of Cr-DNA adducts (Reynolds 2007, 2009, 2012: PMID: 17169990, PMID: 19141647, PMID: 22241526). Consistent with their origin linked to direct Cr-DNA damage, the formation of micronuclei showed a linear dose-dependence on Cr(VI) concentration at noncytotoxic doses.

Additional evidence for mutagenicity of Cr(VI) in animals, from one panel member:

a) The omission/exclusion of the study by Itoh S. *et al*., (1998) is unclear as it provides convincing evidence for Cr(VI) mutagenicity in mice. [Itoh, S., and Shimada, H. (1998) Bone marrow and liver mutagenesis in lacZ transgenic mice treated with hexavalent chromium. *Mutat. Res. 412,* 63-67]
b) Information on fetal genotoxicity of Cr(VI) described on p. 3-296 should be added to the description of animal evidence for the mutagenic MOA: *"One study assessed genotoxicity [measured as the frequency of micronucleated (MN) polychromatic erythrocytes (PCE) in maternal bone marrow and fetal liver and peripheral blood] in mice exposed to Cr(VI) salts during gestation via i.p. injection or oral exposure (De Flora et al., 2006). Fetuses from dams dosed orally via drinking water with sodium dichromate dihydrate (5 or 10 mg/l) or potassium dichromate (10 mg/l) did not have any changes in the frequency of MN PCE compared to controls. In contrast, fetuses from*

dams given a single i.p. injection of 50 mg/kg potassium dichromate or sodium dichromate dihydrate on GD 17 had significantly increased frequency of MN PCE frequency in the liver and peripheral blood. The same pattern was observed in maternal bone marrow. This study suggests that Cr(VI) is genotoxic to fetuses when it reaches target tissues, although bioavailability is poor through the oral route of exposure."

Additional comments from one panel members regarding the mutagenic MOA:

On p. 3-54, lines 10-16, the draft IRIS assessment states: "*This study also did not observe increases in 8-OHdG DNA adducts in the oral cavity or duodenal tissue of mice (Thompson et al., 2011). The absence of oxidatively induced 8-OHdG adducts in mouse GI tissues is consistent with a study by De Flora et al. (2008), which found no increase in these lesions in the forestomach, glandular stomach, or duodenum after female SKH-1 mice were exposed for 9 months via drinking water at concentrations of 1.20 and 4.82 mg Cr(VI)/kg-d. The reason for the lack of oxidative DNA lesions associated with the oxidative stress in these studies is not known."* Similarly, on p. 3-124 (lines 9-10), the draft IRIS assessment states: *The reason for the lack of oxidative DNA lesions associated with the oxidative stress in these studies is not known (In reference to small intestine)*

The utility of a specific form of DNA damage as a biomarker of genotoxic activity is dependent on the persistence of this lesion, which is especially critical for evaluation of chronic exposures. 8-OHdG (8-oxodG) is a short-lived DNA lesion with approximate $t_{1/2}$= 30 min in different human and rodent cells (Cappelli 2000, Lan 2004). Thus, 8-oxodG can be detected immediately after exposure to a sufficiently high dose of an oxidant, but it will become undetectable a few hours later. Thus, in chronic exposures in rodents 8-oxodG cannot serve as a dosimeter of a direct genotoxic damage by Cr(VI), especially considering that as nocturnal animals, rodents consume water primarily during the night. However, no increases in 8-oxodG indicate the absence of strong prooxidant inflammatory responses in small intestine.

1. Cappelli E, Degan P, Thompson LH, Frosina G. Efficient repair of 8-oxo-7,8-dihydrodeoxyguanosine in human and hamster xeroderma pigmentosum D cells. *Biochemistry*. 2000;39(34):10408-10412. PMID: 10956030
2. Lan L, Nakajima S, Oohata Y, Takao M, Okano S, Masutani M, Wilson SH, Yasui A. In situ analysis of repair processes for oxidative DNA damage in mammalian cells. Proc Natl Acad Sci USA 101(38): 13738-13743 (2004). PMID: 15365186

On p. 3-53, the draft IRIS assessment states: "*No human oral exposure studies or human studies of cytotoxicity or cell proliferation specific to the GI tract were identified."*

It is an incorrect statement unless it referred only to *in vivo* exposures. Peterson-Roth E. *et al.* (2005, PMID: 15831465) found that Cr(VI)-treated HCT116 human colon cells displayed induction of p53-independent apoptosis associated with activation of caspases 2 and 7. Cr(VI) also caused the formation of DNA double-strand breaks in these cells as measured by scoring nuclear foci of g-H2AX. Formation of DNA breaks and cytotoxicity by Cr(VI) resulted from

toxic processing of Cr-DNA adducts by mismatch repair. Similar to lung and other types of cells, HCT116 human colon cells showed an abundant formation of Cr-DNA adducts.

On p. 3-54, the draft IRIS assessment states: "*In vitro, it appears that Cr(VI) exposure can result in oxidative stress with minimal or no cytotoxicity, as shown in human colorectal adenocarcinoma Caco-2 cells (Thompson et al., 2012a). Thompson et al. (2012a) measured both 8-OHdG adducts and levels of phosphorylated histone H2AX (γH2AX), a marker of DNA double-strand breaks that could arise from various sources including ROS and/or direct chemical interactions. After 24 hours, cytotoxic concentrations of Cr(VI) increased 8-OHdG and γH2AX levels, while non-cytotoxic concentrations only elevated 8-OHdG, suggesting that oxidative stress could be a mechanism for DNA damage other than double-strand breaks at lower concentrations in in vitro test systems."*

The results of the study by Thompson *et al.* (2012a) on the detection of 8-oxodG are technically unreliable due to a poor specificity of the employed antibody/immunostaining procedure. Immunostaining images in Fig. 4 showed cells with brightly stained perinuclear cytoplasm while nuclear regions appeared negative. Staining for g-H2AX was nuclear, as expected, and these results are not in question.

On p. 3-128 (lines 16-18), the draft IRIS assessment states: "*In vitro, some studies show p53 activation in human lung cells increased with higher Cr(VI) concentrations (Hu et al., 2016) or occurring in vitro and not in vivo (Rager et al., 2017), so the nature of how p53 expression may be affected by Cr(VI) is not understood."*

A paper by Luczak *et al.* (2019, PMID: 31388677) provides an explanation for discrepancies between p53 activation in cell culture models and in vivo – it is linked to the greater role of ascorbate of Cr(VI) metabolism in vivo and suppression of ATM-dependent p53 acetylation.

On p. 4-37, the draft IRIS assessment states: "*Unlike for the RfD, extracellular reduction of Cr(VI) to Cr(III) was assumed negligible for the inhalation route of exposure, and no additional dosimetric factors were applied for pharmacokinetics."*

It is unclear what was the basis for this assumption. Rodents have much more efficient (10-20x) extracellular reduction/detoxification of Cr(VI) in the bronchioalveolar fluids than humans. (PMID: 28759204, Krawic C. *et al.* 2017 and refs. therein; PMID: 36858775).

Figure 3-16. *Key events and mechanistic pathways induced by Cr(VI) exposure that can lead to cancer*

- It overestimates the impact of direct oxidative mechanisms. Studies on mutagenicity and genotoxicity of DNA damage arising during Cr(VI) reduction by ascorbate, cysteine or glutathione all showed the critical role of Cr-DNA adducts (reviewed in Krawic and Zhitkovich, 2023 and Refs. therein).
- It is unclear why Cr-DNA adducts/Direct genotoxicity cannot lead to cytotoxicity and regenerative proliferation.

- Oxidative damage is more likely to be a consequence of chronic inflammation rather than its cause.

In Fig. 3-18 of the draft IRIS assessment:

- It incorrectly depicts oxidative stress by Cr(VI) as arising during high exposure through the formation of O_2^- and H_2O_2 from Cr(V) reduced to Cr(IV).
- High doses of Cr(VI) consume cellular reducers/antioxidants, especially two-electron reducer ascorbate, which leads to the increased yield of Cr(V) due to a shift to the thiol-mediated reduction. Cr(V) can react with cellular H_2O_2 to produce Cr(V)-peroxo species that act as the main oxidants in Cr(VI) reactions (original Refs in review by Krawic C. 2023, PMID: 36858775).

Table C-33 (of the Supplemental Information document):
Inclusion of the results from a study by Peterson-Roth E. (2005 PMID: 15831465) would be helpful.

Figs C-17 through C-20 (of the Supplemental Information document):
These Figures included data from occupational settings that have minimal or no Cr(VI) exposures (tanners, for example) in calculations of the overall risks for GI tract cancers. It would be more informative to restrict these analyses to occupations with well-documented Cr(VI) exposures (chromate production workers, chrome platers, for example).

Conclusions on the mutagenic MOA suggested by one panel member should reference KC #1 section (p. 3-122 of the draft IRIS assessment) on extensive evidence for DNA reactivity of Cr(VI) (presented in p. 3-122 section). As mentioned above, KC #1 section needs to be further strengthened by the inclusion data on cellular and in vitro data on Cr-DNA reactivity.

1.a.1. Additional comments on the mutagenic MOA from one panel member:

"*This evidence suggests that a Cr(VI)-mediated influence on Rad51 may result in modifications to HR, increasing reliance on NHEJ and potentially leading to unrepaired DNA double-strand breaks and increased aneuploidy and genomic instability.*"

This passage is correct in its intent. DNA repair does switch to a more error prone repair, but it is incorrect in detail. Specifically, as the field has progressed, it has become clear that when homologous recombination (HR) begins and fails, as would be the case with Cr affecting Rad51, cells cannot switch to non-homologous end joining (NHEJ), rather they can only switch to MMEJ (microhomology-mediated end joining) or SSA (single strand annealing) repair. These more minor repair pathways are key to resolving failed HR repair and are actually less reliable and more destabilizing than NHEJ repair. This passage should be revised to reflect the correct switch.

The draft IRIS assessment does not discuss or apparently allow for Cr(VI)-induced DNA double strand breaks to cause cytotoxicity. It is well understood in the field that a single unrepaired DNA double strand break can induce cytotoxicity. Thus, one would expect cytotoxicity to follow breaks. The draft IRIS assessment has many passages assigning the cytotoxic effects to oxidative damage. Here are a few (there are more):

- Page xvi, lines 6-7 "…mechanistic studies support the involvement of oxidative stress in Cr(VI)-induced cytotoxicity…", Pages 3-35-3-37
- Page 3-111, lines 26-27 "…oxidative stress and free 26 radical-induced cytotoxicity and DNA damage;…"
- Page 3-122, lines 35-36 "...Oxidative stress induced by Cr(VI) exposure appears to lead to several toxicity pathways causing cytotoxicity,..."
- Page 3-123, 'lines 1-2 "…Cr(IV) that produce reactive oxygen species, which can cause cytotoxicity and directly damage 1 intracellular molecules including DNA,…
- Page 3-124, lines 21-22 "…cancer cell lines to study oxidatively induced DNA damage and cytotoxicity."
- Page 3-124, lines 25-26 "In addition to oxidative stress initiating cytotoxicity and DNA damage following Cr(VI) exposure,…"
- Page 3-125, lines 17-20 "Overall, there is a consistent, coherent, and biologically plausible evidence base available to 17 describe the intracellular reduction and redox imbalance, oxidative stress, and cellular oxidative 18 damage due to free radical generation caused by Cr(VI) exposure, potentially contributing to 19 cytotoxicity, genetic damage, and cell proliferative signaling pathways."

However, at the same time, the draft IRIS assessment acknowledges DNA repair is inhibited including DNA double strand break repair. For example, page 3-127, lines 9-24 discuss studies to this effect. Yet, the draft IRIS assessment does not mention that a meaningful proportion of observed cytotoxicity may result from suppressed repair of breaks and other types of DNA damage. This aspect has important ramifications and should be considered.

One panel member also suggested that conclusions in Cancer mode-of-action summary be toned down:

On pages 3-139 and 3-140, the draft IRIS assessment states: "*High levels of cytotoxicity can lead to the detection of increased DNA damage in some test systems. For this reason, the interpretation of genotoxicity evidence from chemicals inducing excessive toxicity includes efforts to determine whether increases in genotoxicity are potentially secondary to cytotoxicity. For the Cr(VI) in vivo oral exposure database, there is not enough evidence to determine whether and to what extent Cr(VI)-induced genotoxicity might be the result of (secondary) cytotoxic DNA damage in the GI tract. Most notably, while many of the animal studies examining the most relevant genotoxicity endpoints did not detect substantial evidence of genotoxicity at doses that also caused histological effects in the GI tract, including diffuse epithelial/crypt cell hyperplasia and degenerative changes in the villi (vacuolization, atrophy, and apoptosis), one study did observe statistically significantly increased micronuclei in villous cells from animals exposed to doses that similarly induced villous atrophy and apoptosis. Because no studies were available that specifically examine the presence or absence of*

genotoxicity in the GI tract as the MTD was approached and exceeded, this uncertainty cannot currently be addressed."

This passage is troublesome for a few reasons: 1) It asserts unidirectionality to cytotoxicity and DNA damage (i.e., "*High levels of cytotoxicity can lead to the detection of increased DNA damage in some test systems*"). This may be true, but at the same time low levels of some DNA damage can lead to cytotoxicity and this possibility, that the observed cytotoxicity results from DNA damage, is not considered. 2) The claim is that: "*Most notably, while many of the animal studies examining the most relevant genotoxicity endpoints did not detect substantial evidence of genotoxicity…*". There are subjective qualitative issues with this claim. For example, the phrase "most relevant genotoxicity endpoints". It is unclear that the handful of endpoints measured as truly the "most" relevant as opposed to simply being relevant. DNA single strand breaks were not measured, and they are likely relevant along with other damage not considered. Similarly, "many" is a subjective word. It is unclear that many studies examined each endpoint. For example, only two animal studies by the same authors measured gamma-H2A.X, and of these each was a different time point so really only 1 study measured this endpoint. 3) Was the DNA damage work done robustly? The gamma-H2A.X work was not done to a high-quality level as noted above (See 3.a.3. Discuss the uncertainty and limitations in gamma-H2A.X studies as was done for other experimental endpoints).

Toning this language down to something more like the following (bolded font for clarity):

"*High levels of cytotoxicity can lead to the detection of increased DNA damage in some test systems. For this reason, the interpretation of genotoxicity evidence from chemicals inducing excessive toxicity includes efforts to determine whether increases in genotoxicity are potentially secondary to cytotoxicity. For the Cr(VI) in vivo oral exposure database, there is not enough evidence to determine whether and to what extent Cr(VI)-induced genotoxicity might be the result of (secondary) cytotoxic DNA damage in the GI tract. Most notably, while* ***some*** *animal studies examining* ***some relevant*** *genotoxicity endpoints* ***(insert various endpoints here)*** *did not detect substantial evidence of genotoxicity at doses that also caused histological effects in the GI tract, including diffuse epithelial/crypt cell hyperplasia and degenerative changes in the villi (vacuolization, atrophy, and apoptosis), one study did observe statistically significantly increased micronuclei in villous cells from animals exposed to doses that similarly induced villous atrophy and apoptosis.* ***DNA damage may result in cytotoxicity and not all relevant DNA lesions were measured in these studies, which confounds interpretation of the outcomes.*** *Because no studies were available that specifically examine the presence or absence of genotoxicity in the GI tract as the MTD was approached and exceeded, this uncertainty cannot currently be addressed.*"

Relative to the cytotoxicity discussion:

A fundamental question in the MOA is whether cytotoxic mechanisms or genotoxic mechanisms prevail in the MOA for Cr(VI). The cytotoxic implications are discussed throughout the document as are the DNA damage implications. A section that clarifies what is known about them occurring together or in sequence would be useful and enhance clarity. It would be helpful

to have a specific discussion about whether cytotoxicity followed DNA damage and oxidative stress or if DNA damage and oxidative stress followed cytotoxicity.

Currently, this type of information is scattered throughout the draft IRIS assessment and where the analyses appear they are appropriate, but the weight of evidence aspect becomes less clear. It is hard to ascertain how many studies show oxidative damage or DNA damage in the absence of cytotoxicity and, conversely, how many studies show cytotoxicity in the absence of oxidative damage or DNA damage.

Also missing is a qualitative analysis of the strengths and limitations of the data. How rigorous were the analyses of DNA damage, oxidative damage and cytotoxicity in this collection of studies? Were the assessments robust and did they actually measure cell death to show an absence of cell death? Were the DNA damage assays robust and varied (different types of DNA damage and sensitive measures) to show an absence of DNA damage? For example, a lack of 8-hydroxy-2′-deoxyguanosine (8-OHdG) lesions does not mean there are no DNA double strand breaks, and a lack of DNA double strand breaks does not mean other DNA lesions do not occur.

Such a section would help the reader interpret the passage, on pages 3-139 and 3-140, where the draft IRIS assessment states:

> "*High levels of cytotoxicity can lead to the detection of increased DNA damage in some test systems. For this reason, the interpretation of genotoxicity evidence from chemicals inducing excessive toxicity includes efforts to determine whether increases in genotoxicity are potentially secondary to cytotoxicity. For the Cr(VI) in vivo oral exposure database, there is not enough evidence to determine whether and to what extent Cr(VI)-induced genotoxicity might be the result of (secondary) cytotoxic DNA damage in the GI tract. Most notably, while many of the animal studies examining the most relevant genotoxicity endpoints did not detect substantial evidence of genotoxicity at doses that also caused histological effects in the GI tract, including diffuse epithelial/crypt cell hyperplasia and degenerative changes in the villi (vacuolization, atrophy, and apoptosis), one study did observe statistically significantly increased micronuclei in villous cells from animals exposed to doses that similarly induced villous atrophy and apoptosis. Because no studies were available that specifically examine the presence or absence of genotoxicity in the GI tract as the MTD was approached and exceeded, this uncertainty cannot currently be addressed.*"

Currently, it is difficult for the reader to evaluate the claims as: 1) the possibility that the observed cytotoxicity results from DNA damage were not considered. 2) The claim is that: "*Most notably, while many of the animal studies examining the most relevant genotoxicity endpoints did not detect substantial evidence of genotoxicity…*" is very difficult to examine the veracity of this claim. One cannot tell without reading the whole document with a scorecard, which studies are referred to and the specific endpoints are also not listed here for the reader to agree or disagree. Were there many? Again, it is hard to ascertain without a section discussing them together. 3) It's hard to do a quality assessment as it's difficult to find the discussion points of these papers. The gamma- H2A.X work was not done to a high-quality level as noted above (See 3.a.3. Discuss the uncertainty and limitations in gamma-H2A.X studies as was done for

other experimental endpoints). Adding a section discussing cytotoxicity and genotoxicity together could be pointed to and would clarify this important passage and this key point more effectively.

Relative to the lung inflammation discussion:

A fundamental question for the MOA in the lung is whether inflammatory mechanisms or genotoxic mechanisms prevail in the mode of action for Cr(VI). The inflammatory implications are discussed throughout the draft IRIS assessment as are the DNA damage implications. What is missing is a section that clarifies what we know about them occurring together or in sequence. It would be helpful to have a specific discussion about whether inflammation followed DNA damage and oxidative stress or if DNA damage and oxidative followed inflammation. Currently, this type of information is scattered throughout the draft IRIS assessment and where the assessments appear they are appropriate, but the weight of evidence aspect becomes less clear. It's hard to ascertain how many studies show oxidative damage or DNA damage in the absence of inflammation and, conversely, how many studies show inflammation in the absence of oxidative damage or DNA damage.

Also missing is a qualitative assessment of the strengths and limitations of the data. How rigorous were the assessments of DNA damage, oxidative damage and inflammation in this collection of studies? Were the assessments robust and did they actually measure cell death to show an absence of cell death? Were the DNA damage assays robust and varied (different types of DNA damage and sensitive measures) to show an absence of DNA damage? Such a section would help inform the reader for the decisions made.

Relative to the micronuclei discussion:

Micronuclei are used as a tool to measure chromosome aberrations and aneuploidy. Assessing micronuclei is a direct and less sensitive measure than considering chromosomes directly by counting the number and assessing the structure in a chromosome aberration assay. For Cr(VI), data from cell culture studies show a robust clastogenic response when considering chromosomal changes directly and a much less sensitive and robust response when considering micronuclei. The studies underlying the draft IRIS assessment rely heavily on the indirect measures of the micronucleus assay and the draft IRIS assessment should note this reduced sensitivity in its uncertainty consideration. The clastogenic effect is likely much higher than reported in the micronucleus assay and some negative studies may be false negatives as result of this lowered sensitivity.

Relative to future considerations:

The current practice seems to be to put all cell line studies in a bin of 'informing mechanism' with no regard for cell line quality. Hence, low quality cell lines (for determining mechanism) like A549 and MOLT4 are referenced as examples. But there are data in more robust cell lines. At a minimum, the draft IRIS assessment needs to also consider the limitations of cell culture models. Not all cell culture models are the same and depending on the question some are quite limited and those limitation should be noted and weighed. For example, consider the point

mentioned above in section 3.a.1. Avoid including data/conclusions from cell lines unable to measure the effect considered. A cell line like BEAS-2B with functionally inactive p53 should not be considered for studies of p53 function. Another example, would be thinking about GI tract effects - would chromosome instability effects be more relevant in a cell line like Caco-2 that are inherently chromosomally unstable, but are from a tumor derived from the gut versus a chromosomally stable cell line from another organ? It's not a simple answer and likely involves consideration of both with their strengths and limitations noted. The EPA should reflect and consider its procedure for handling cell line-driven data in a more robust manner.

Along those lines, as the veracity of cell lines has come into question and authentication of cells becomes required, the EPA should start to consider how to classify cell culture studies as low, medium and high confidence to help interpret the data. Historically, cell lines were not authenticated and yet that data has value, perhaps these become low or medium confidence studies. There are databases that track cell lines with histories of cross contamination with other cell lines (such as Hela cells) and perhaps data from those cell lines become uninformative.

Technology is rapidly progressing into single cell analysis, which will inform in very different ways. It will become increasingly clear that compartments currently treated as one unit (e.g., crypt and villus) in this example are experiencing an array of cell outcomes within the compartment. Hence, something that appears negative will actually be revealed to have a subset of highly positive outcomes, whose signal was swamped out when considered together with the negative outcomes. Such an aspect does not apply to the current report as there were no studies of single cell analysis. However, single cell analysis studies are being performed for various chemicals and they will start to become a part of other future reports. The EPA should begin discussing now how to best incorporate these new tools and will the absence of these new tools then create additional uncertainty for the interpretation of chemicals that lack such data. It is an important consideration for the future.

<u>Comments on Potential nonmutagenic MOA from one panel member</u>:

Cytotoxicity/Regenerative proliferation

Hyperplasia consistent with regeneration following cell injury has been reported following oral exposures in the small intestine of mice and rats and following inhalation exposures in the lung in rats. Hyperplasia has not been observed in the oral cavity of rats (site of tumorigenesis in rats) following Cr(VI) exposures. However, no statistically significant or dose-dependent changes were found in mitotic or apoptotic indices in tissue regions with increased crypt length, area, and number of crypt enterocytes. It is possible that the observed limited hyperplasia was a manifestation of regenerative responses although other causes cannot be ruled out (diminished cell death of enterocytes, increased differentiation of progenitor cells into villus cells, for example). Increased proliferation could be viewed as an alternative mechanism for indirect induction of mutations due to higher rates of cell division. This carcinogenic process would exhibit a strongly sublinear or threshold-type dose dependence, as it relies on the induction of cell death and small doses would not kill cells. The extent of hyperproliferation in chromium-

exposed groups was modest, and considering the overall very high rate of cell division in the small intestine, it is hard to see how somewhat faster replication would provide dramatically more spontaneous mutations required for cancer development.

Main counterarguments for the importance of hyperplasia/regenerative proliferation in Cr(VI) carcinogenicity:

1. Regenerative proliferation as a MOA for Cr(VI) is currently a theoretical concept with no direct experimental data demonstrating its role in carcinogenic activity of Cr(VI) or the formation of genetic changes (cancer is a genetic disease) in small intestine, oral cavity or lung cells.
2. The shape of dose-response curves for tumors in mouse small intestine was linear (male mice) or linear/supralinear (female mice) and not sublinear, as it would be expected for carcinogenesis via cytotoxicity-driven hyperproliferation.
3. Epidemiological studies also found a linear dose-dependence of lung cancer risks in occupational exposures:

Gibb, H.J., Lees, P.S., Pinsky, P.F., and Rooney, B.C. (2000) Lung cancer among workers in chromium chemical production. *Am. J. Ind. Med. 38,* 115-126.

Proctor, D.M., Suh, M., Mittal, L., Hirsch, S., Valdes Salgado R., Bartlett, C., Van Landingham, C., Rohr. A., Crump, K. (2016) Inhalation cancer risk assessment of hexavalent chromium based on updated mortality for Painesville chromate production workers. *J. Expo. Sci. Environ. Epidemiol*., *26*, 224-231.

4. Hyperplasia was not detected in the oral cavity of rats where Cr(VI) also caused tumors.
5. Modest increases in proliferation of crypt cells would not be expected to provide a significant boost in their mutation burden. Independence of age-dependent accumulation of mutations in tissues with dramatically different proliferation kinetics demonstrates that the rate of cell proliferation makes only a small contribution to the overall mutation rates in tissues (Fig. 1D in Ren P. *et al.* 2022). For example, mutation rates in small intestine crypt cells were similar to those in hepatocytes. Despite extremely high rates of proliferation during spermatogenesis, sperm cells had dramatically lower mutation rates than any other tissue. Hematopoietic stem cells and progenitors had mutation rates comparable to those in frontal cortex neurons (nonproliferative cells), prostate epithelium (slowly proliferating) and lower than in the lung, adipose tissue or liver (slowly renewing tissues). Despite dramatically lower proliferation rates in prostate relative to small intestine, prostate is the most frequent cancer among men in the US whereas small intestine is not even in the top 10 most common sites for human cancers.

The panel discussion relative to mode of action also included the detailed comments of one panel member as provided below.

Relevant to the MOA for the small intestinal tumors in mice, one panel member agreed that the information presented in the draft IRIS assessment (Section 3.2.3.2, starting on p. 3-93; p. 3-139, lines 10 – p. 3-140, line 11) about the limitations of the animal studies that evaluated genotoxicity from oral (e.g., drinking water) exposure, including the studies with negative

results, supports the conclusion that these studies are of "low confidence." The panel's discussions about problematic issues with these studies provided further support for the conclusion that they are of low confidence for multiple reasons other than that the Maximum Tolerated Dose was not used.

- Table 3-19 on p. 3-97. System/Exposure information is missing for Thompson *et al.* (2017). This is a **Tier 1**: Necessary Revision.

One panel member agreed with the draft IRIS assessment's conclusion that "there is evidence for regenerative hyperplasia as a key event for tumors of the small intestine in mice" (p. 3-134, lines 7-8) and that "multiple modes of action for tumor formation in the mouse small intestine could be occurring in parallel…" (Appendix D-31, lines 4-5).

The panel member also agreed with the draft IRIS assessment's conclusion (p. 3-134, lines 18-19) of a mutagenic MOA for oral tumors in the rat and that "there is no evidence to conclude regenerative hyperplasia is involved in the tumorigenic process in the rat oral cavity." However, it is stated on p. D-32, line 3, that: "Tumors of the rat oral cavity did not have a proposed mode of action…" This statement needs to be revised to say that it was concluded that rat oral cavity tumors have a mutagenic MOA (**Tier 1:** Necessary Revision).

Regarding the presentation of the MOA evaluation in general, one panel member had the following specific 3-comments:

- The panel member suggested that it be explicitly stated that the 10 key characteristics of carcinogens from Smith *et al.* (2016) are mentioned in the ORD Staff Handbook for Developing draft IRIS assessments (2022) as an approach that "provide[s] a systematic method for identifying, organizing, and summarizing the available mechanistic studies for analysis and interpretation. for organizing MOA data." This will clarify why the Smith *et al.* (2016) approach is emphasized in the draft IRIS Cr (VI) assessment.

The IRIS Handbook also mentions that "there are other variations of approaches to organizing, analyzing, and synthesizing mechanistic information that have similarities to those discussed here, and additional examples will be developed as the field advances." However, it does not specifically mention the hallmarks/enabling characteristics of cancer from Hanahan (2022) and Hanahan and Weinberg (2011) that are discussed in the draft IRIS Cr (VI) assessment. The panel member suggested that it be clarified whether the Hanahan (2022) and Hanahan and Weinberg (2011) hallmarks/enabling characteristics are relevant specifically to the tumor data for Cr (VI) or if they are to be used by IRIS for cancer hazard identification and MOA analysis in general (**Tier 1:** Necessary Revisions).

- Table 3-20. For each key event where relevant, suggest stating whether the effects mentioned were observed in humans, in laboratory animals, and/or in *in vitro* studies (**Tier 2:** Suggested Revision).
- p. 3-126. Line 31-32. "15.4%" was omitted, and the sentence should be revised to "at two or more loci in 78.9% of lung cancers with chromate exposure compared to 15.4% of lung cancers without chromate exposure (**Tier 1:** Necessary Revision).

Additional individual panel member detailed comments regarding mode of action:

The conclusions related to high dose exposure, in general, seemed reasonable to one panel member. However, three panel members believed that additional examination is warranted evaluating the carcinogenic risks at very low to ultra-low doses. One of these panel members described how key steps in the transformation, uptake and detoxication of Cr(VI) and its derived reactive species may result in deviations from linearity in response. For example, diffusion within the extracellular space (across the mucous layer) should be much slower at very low doses given that it should be related to concentration. This combined with gastric emptying would likely substantially reduce the concentration of Cr(VI) traversing the mucous layer. Secondly, the ability of gastric fluid and dietary contents to reduce Cr(VI) to Cr(III) prior to uptake by the cell at experimental concentrations has been discussed. However, how this process would proceed at very low doses where the reducing capacity greatly exceeds the exposure dose has not been thoroughly examined. As an example, studies by Donaldson and Barreras (1966) where conducted at very low doses (20 ng Na_2CrO_4) were administered to human volunteers orally and directly into their duodenum may provide useful insights. The pH in the intestine has been reported to be lower closer to the cell wall than in the lumen (Marletta, 1989) suggesting that the reduction of Cr(VI) would be more efficient than estimated by using average intestinal pH values. Another key step is the uptake of Cr(VI) by phosphate and sulfate transporters. At elevated doses, Cr(VI) is able to readily enter the cells. However, at very low doses, phosphate and sulfate would be at much higher concentrations and may compete for uptake by the receptor. When the Cr(VI) dose becomes very low, one would expect that phosphate and sulfate would act as competitive inhibitors reducing or greatly reducing the ability of Cr(VI) to enter the cell. While the review mentions that uptake is a competitive process, one panel member didn't believe that this potential limiting step has not been discussed in the toxicological review. Lastly, the reduction of Cr(VI) to Cr(III) by cellular antioxidants such as ascorbate and GSH has been well described. This reduction is correctly described as an activation step. However, these antioxidants also act to reduce and inactivate reactive species formed during this process. As a result, it would seem that at very low doses of Cr(VI) where the antioxidants and antioxidant enzyme capacity greatly exceed the Cr(VI), Cr(V) and Cr(IV) concentrations, these reactive species as well as derived reactive oxygen species should be efficiently reduced to less reactive or inactive species. The intracellular reduction of Cr(VI) at very low doses should efficiently result in Cr(III).

Cr(III) is an unusual and enigmatic compound. While its role as an essential nutrient and beneficial supplement is an area of scientific debate, it has been recognized by the National Academy of Sciences as being an essential nutrient and is widely accepted as such. For example, it is found in many multi-vitamins. While poorly absorbed, Cr(III) has been shown to have beneficial effects in livestock and is a commonly used supplement in feed. It is also used in some situations at elevated doses as a nutritional supplement in humans. Cr(III) has been shown to exhibit some genotoxic and mutagenic effects, but general toxicology studies have indicated that it has low to moderate toxicity, and chromium picolinate, a bioavailable form of Cr(III), was largely negative for both neoplastic and non-neoplastic effects in an NTP 2-year cancer bioassay, even when tested at doses of up to 50,000 ppm in the diet. One would expect that at very low doses of Cr(VI), the Cr(III) that is formed in the cell would fall within background levels that would exhibit no effect and could potentially even be beneficial.

Given the above information, the one panel member believes that an additional discussion and evaluation of Cr(VI)'s mode of action in the very low dose range is warranted.

Additional individual panel member provided detailed comments on mode of action:

One panel member had the following comments: *EPA has demonstrated genotoxicity hazard but has not conducted a MOA analysis that demonstrates a mutagenic MOA for carcinogenicity. Moreover, EPA's MOA conclusion is far from representing scientific consensus (i.e., "settled science") as scientists at other notable agencies and elsewhere have concluded that an alternate MOA is best supported by the weight of available relevant scientific evidence.* Despite EPA summarizing a great deal of information in attempting to establish a mutagenic MOA, the primary focus of EPA is more simply on demonstrating genotoxicity hazard. That is, EPA's focus is primarily determining if, or documented that, Cr(VI) has been demonstrated to have the ability induce genotoxicity (if not mutagenicity).[6] This is consistent with EPA's February 15, 2023 presentation to the Science Advisory Board (SAB) indicating that a focus on mutagenic evidence was a primary goal of the draft (slide 43). Following what is a genotoxicity hazard assessment, just one part of a MOA analysis (TCEQ 2015, EPA 2005a),[7] EPA simply relies on genotoxicity as a plausible/possible carcinogenic MOA, stating, "In conclusion, there is consistent and coherent evidence that a mutagenic MOA for Cr(VI)-induced carcinogenesis is biologically plausible and relevant to humans" (similar statement on p. 3-130, lines 5-6). The primary basis for EPA's conclusion as to the carcinogenic MOA was simply a genotoxicity assessment... genotoxicity in some tissues = biological plausibility (i.e., a possible MOA) = demonstrated mutagenic MOA in target tissues. EPA presented evidence for Cr(VI)-induced genotoxicity, but no Cr(VI)-specific evidence for establishing a mutagenic MOA past that.[8] As Dr. Toby G. Rossman, a genotoxicity/ mutagenicity expert peer reviewer who participated in the *Peer Review Workshop for EPA's draft Toxicological Review of Hexavalent Chromium*, points out… "Standard genotoxicity assays were not designed to inform specific modes of tumor induction… In summary, standard genotoxicity assays from hazard identification exercises cannot be used to establish a mutagenic MOA…" (pp. A-55 and A-56, July 6, 2011, post-meeting comments). The lack of Cr(VI)-specific evidence for establishing a mutagenic MOA is clearly evident for the cancers made basis for the draft SFo,[9] where EPA's MOA conclusion relies on two factors: (1) not all Cr(VI) being reduced extracellularly prior to absorption; and (2) Cr(VI) exhibiting genotoxicity (see p. 3-140, lines 20-25). EPA has conducted no analyses that show or suggest causation or dose-response/temporal concordance between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue, although EPA recognizes the

[6] EPA states on p. 3-138 (lines 5-6), "…evidence of transmissible and permanent genetic alterations have been prioritized for the analysis of a mutagenic MOA…"

[7] Two key weight of evidence determinations are involved in applying the EPA (2007) framework. They generally concern the critical underlying questions of interest: (1) Does the carcinogen demonstrate mutagenic activity?; and (2) Is the carcinogen operating via a mutagenic MOA in the cancer target tissue? (TCEQ 2015, p. 162).

[8] As recognized in public comments (dated December 19, 2022) by the American Water Works Association (pp. 1-2).

[9] "Less clear" in the following EPA statement seems an understatement, "The evidence for a mutagenic MOA following oral exposures is less clear" (p. 3-139, line 10); it is far from clear in my opinion.

importance of conducting such analyses for supporting a mutagenic MOA.[10] Indeed, an important criterion in EPA (2005) is "similar dose-response relationships for tumor and mode of action-related effects" (Section 2.3.5.4 *Judging Data*) or "dose-response concordance" (p. 2-45), and a demonstration of temporality is the most basic of the Hill criteria (p. 2-45 of EPA 2005a).[11] Based on the study data discussed in the draft, there was no Cr(VI)-specific data that demonstrate a connection between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue (contrary to the solid arrows in Figures 3-16 and 3-18 and the statement on p. 3-137 lines 22-23). For example, the increased micronuclei (MN[12]) detected in blood and exfoliated nasal and oral epithelial cells demonstrate no such connection that Cr(VI)-induced genotoxicity is, or likely is, responsible for the initiation of cancer in target tissues (e.g., cited by EPA on p. 3-138, lines 9-10). Moreover, Dr. Rossman comments that MN arise from malsegregation and not DNA strand breaks at lower Cr(VI) concentrations, and aneugenesis is caused by alterations in proteins (not DNA) and has thresholds.[13] Demonstrating mere biological plausibility is not tantamount to demonstrating a MOA. Indeed, EPA acknowledges that, "the specific role of Cr-species and Cr-induced DNA lesions in the toxicity and carcinogenicity of Cr(VI) has not yet been conclusively established" (p. 3-122, lines 23-24), although "conclusively" appears to be an unnecessary caveat in regard to the carcinogenic MOA as it appears from the draft that although Cr-species and Cr-induced DNA lesions have been shown, no role has been established for any form of Cr(VI)-induced genotoxicity in the target tissue carcinogenicity of Cr(VI). Again, there are no dose-response/temporal analyses to even show concordance between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue, so "Is the key event associated with precursor lesions?" (EPA 2005a, p. 2-44) cannot be answered in the affirmative in this case. Consequently, the draft must rely on assumption and speculation rather than such analyses (e.g., dose-response/temporal analyses) in an attempt to draw a connection to explain the MOA operating in Cr(VI) target tissues.[14] This appears to be the reason that the draft concentrates on genotoxicity hazard assessment to *draw the MOA conclusion at the end of the genotoxicity hazard assessment* (p. 3-111, lines 3-4); assembling data to most simply show that Cr(VI) has been shown to be capable of inducing genotoxicity (at least under certain conditions). To then assume that genotoxicity must therefore be the carcinogenic MOA operating in target tissues requires a leap of faith that some are not

[10] EPA states, "…evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA" (p. 3-139, lines 26-28).

[11] As one example of such analyses for an alternate MOA, see Figure 5 of Thompson et al. (2018).

[12] Micronuclei indicate aneuploidy or the presence of chromosomal aberrations (p. 3-89, lines 3-4).

[13] Comment from Dr. Toby G. Rossman, a genotoxicity/mutagenicity expert peer reviewer who participated in the *Peer Review Workshop for EPA's draft Toxicological Review of Hexavalent Chromium* (see p. A-56, July 6, 2011 post-meeting comments).

[14] Table 3-122 ("gene and chromosomal mutation" row on p. 3-116) states "Bulky Cr-DNA lesions lead to replication fork stalling and DNA double-strand breaks, which can become fixed mutations if not efficiently repaired or targeted for cell death by apoptosis. Some of these mutations may confer a growth advantage, leading to a clonal outgrowth of the mutated cells and tumorigenesis, a process that is more likely to occur in rapidly proliferating cells." This is speculative and is not tantamount to data allowing a solid arrow to tumors in Figure 3-16 (p. 3-113); that is, it is unknown if this is the process initiating Cr(VI)-induced tumors, so as such it is speculation, Figure 3-16 should not have solid arrows between these speculative events and the tumors. Speculating a mutagenic MOA is also revealed in important text such as [*emphasis added*], "…the ability of Cr(VI) to reach the crypts (where stem cells reside), *which could give rise to* cytotoxicity as well as fixed *mutations*…" (p. 3-121, lines 2-3).

willing to make due to both a lack of supporting data in the present case as well as it being contrary to good guidance on evaluating the potential for a mutagenic MOA (e.g., EPA 2007, TCEQ 2015). "The determination that a chemical carcinogen is capable of producing mutation is not sufficient to conclude that it causes specific tumors by a mutagenic MOA" (EPA 2007, TCEQ 2015). *The apparent lack of data (e.g., dose-response/temporal concordance analyses) that demonstrate a connection between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue (e.g., small intestine tumors in mice serving as the basis for the draft SFo) precludes any demonstration or conclusion that the carcinogenic MOA is a mutagenic one*. It cannot be confidently/scientifically assumed that… genotoxicity in some tissues = biological plausibility/possibility = demonstrated mutagenic MOA in target tissues (e.g., mouse duodenum). This is too low of a bar as it is not a scientifically robust one (e.g., EPA 2007, TCEQ 2015). Nevertheless, this hypothesis or assumption, unsupported by EPA-acknowledged analyses relevant to demonstrating a MOA (some mentioned above), was carried forward into Section 3.2.3.4 (*Mode-of-Action Integration of Evidence for Carcinogenesis*) where it serves as the basis for EPA's assumed carcinogenic MOA. Relevant evidence is discussed by Dr. Toby G. Rossman, a genotoxicity/mutagenicity expert, in the *Peer Review Workshop for EPA's draft Toxicological Review of Hexavalent Chromium* post-meeting comments, who states, "there is no evidence for mutagenicity at the target tissue at all." Concurring is Dr. Sam M. Cohen, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center, stating, "In totality, there is clearly no evidence for a genotoxic response in the duodenum following oral administration of Cr(VI)" (p. 1 of EPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2002). Dr. Rossman has similarly concluded as the panel member and other organizations/agencies have that "the evidence for a mutagenic MOA is weak" (pp. A-57 and A-58, July 6, 2011 post-meeting comments), with Dr. Cohen stating, "There is no question that the mode of action for Cr(VI)-induced small intestinal tumors in mice is cytotoxicity with regenerative proliferation leading to development of neoplastic lesions (adenomas and carcinomas)" (p. 2 of EPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2022). This cytotoxicity-induced regenerative hyperplasia MOA, however, is supported by evaluations of dose-response concordance and temporal concordance (e.g., see Figure 5 of Thompson *et al.* 2018 and Table 9 of Haney 2015c).

Lastly, it is simply noted that if Cr(VI) were to have a mutagenic MOA, then a carcinogenic response might well be expected in mouse and rat tissues demonstrated to have absorbed and retained significant Cr(VI) doses across all dose groups and as early as by day 6 of exposure (e.g., kidney, liver per Appendix J of NTP 2008).[15] Since significant absorption of a mutagenic carcinogen by multiple tissues of a species outside the POE and very early in a chronic study (by day 6) should result in tumors at multiple sites in that species (both inside and outside the POE) if mutation is the key event initiating carcinogenesis, EPA should explain this apparent discrepancy (i.e., carcinogens that have a mutagenic MOA and are distributed systemically with

[15] For example, at the three higher doses/water concentrations (i.e., 53.7, 172, and 516 mg SDD/L), by day 182 of exposure the concentrations of Cr in the rat kidney (5.464-15.262 mg/kg; Table J1 of NTP 2008) had markedly surpassed those in the rat oral tissue (1-5 mg/kg; Table 2 of Kirman et al. 2012) at day 90 of exposure to essentially the same water concentrations (i.e., 60, 170, 520 mg SDD/L). Additionally, at the three higher doses/water concentrations, concentrations of Cr in the rat liver at 90 days of exposure were higher than those in the rat oral tissue at 90 days (Table 2 of Kirman et al. 2012). Similarly, at day 182 of exposure to the three higher water concentrations, the concentrations of Cr in the rat liver (1.568-6.650 mg/kg; Table J1 of NTP 2008) were higher than those in the rat oral tissue at 90 days of exposure (1-5 mg/kg; Table 2 of Kirman et al. 2012) to essentially the same water concentrations.

significant absorption by a variety of tissues should be multisite carcinogens that also produce tumors outside the POE; **Tier 2** suggestion).

By contrast, the available data have led the World Health Organization (WHO 2020), Health Canada (2016), the Food Safety Commission of Japan (FSCJ 2019), the Texas Commission on Environmental Quality (TCEQ 2016), and others to recently conclude that the weight of evidence for the oral route supports a different, threshold MOA. Thus, EPA's carcinogenic MOA conclusion as it applies to the oral route is far from "settled science,"[16] which at a minimum should be explicitly acknowledged in the main assessment (Tier 1 necessary revision) in the interest of full transparency, giving rise to theoretical excess risk estimates (through use of the draft SFo) with which other agencies (e.g., WHO, Health Canada, FSCJ, TCEQ) and researchers would disagree. As the carcinogenic MOA is an obvious major area of uncertainty for the draft SFo, its exclusion from more detailed discussion in the uncertainty section of the main draft IRIS assessment (Section 4.3.5) is neither transparent nor acceptable (Tier 1 necessary revision for inclusion of such a discussion). Other organizations, regulatory agencies and researchers have reasonably concluded that the carcinogenic MOA has not been demonstrated to be mutagenic and that the scientific weight of evidence best supports a different carcinogenic MOA for oral exposure to Cr(VI). More specifically, the recent assessment by the World Health Organization (WHO 2020) adopted a threshold MOA for Cr(VI)-induced carcinogenicity via oral exposure, indicating that weight-of-evidence analyses support a threshold MOA involving hyperplasia in the small intestine as a key precursor event to tumor development (p. 24 of WHO 2020).[17] Add to this Health Canada (2016), which evaluated the carcinogenic MOA weight of evidence and indicated that the carcinogenic MOA analysis supports hyperplasia as a key precursor event to tumor development and a threshold approach for the risk assessment for ingested Cr(VI) such that diffuse hyperplasia of the small intestine was used by Health Canada as the most sensitive endpoint and precursor to tumor formation protective of both non-cancer and cancer effects (p. 59 of Health Canada 2016). The Food Safety Commission of Japan (FSCJ) has also adopted a threshold MOA for Cr(VI)-induced carcinogenicity via oral exposure, stating, "The mechanism of small intestinal tumors in mice is considered as follows: Continuous damage to mucosal epithelium in the small intestine by long-term exposure to Cr(VI) induces the hyperplasia in the crypt of small intestine, which would lead to the formation of tumor" and "Therefore, FSCJ chose the pre-cancerous lesion as the critical endpoint to specify TDI" (pp. 56 and 57 of FSCJ 2019). Consistent with the carcinogenic MOA conclusions by the WHO, Health Canada, and FSCJ, others have also evaluated the available scientific evidence and reasonably concluded that the weight of evidence does not support a mutagenic MOA but rather supports a threshold MOA

[16] For example, EPA states, "The evidence for a mutagenic MOA following oral exposures is less clear" (p. 3-139, line 10), and, "the specific role of Cr-species and Cr-induced DNA lesions in the toxicity and carcinogenicity of Cr(VI) has not yet been conclusively established" (p. 3-122, lines 23-24).

[17] For example, "Using the newer, high-quality data from chronic drinking-water carcinogenicity studies for Cr(III) and Cr(VI) (NTP, 2008a, b), and weight-of-evidence analyses supporting a threshold MOA (Health Canada, 2016), a GV of 50 µg/L remains valid (Moffat et al., 2018). The NTP (2008b) study allows a risk assessment of Cr(VI) in drinking-water that considers both cancer and noncancer effects, and provides evidence to support an MOA involving hyperplasia in the small intestine as a key precursor event to tumour development. Thus, a GV for Cr(VI) in drinking-water considering hyperplasia as the most sensitive end-point and precursor of tumour formation is protective of both cancer and noncancer effects. The current GV of 50 µg/L (total chromium) is therefore considered to be adequately protective of health and is retained, with the previously allocated 'provisional' status removed."

(e.g., Thompson *et al.* 2013, Haney 2015c/TCEQ 2016). EPA's conclusion as to the carcinogenic MOA as well as subsequent EPA modeling choices/assumptions (i.e., linear low-dose extrapolation) are at odds with these recent carcinogenic MOA determinations by other organizations, regulatory agencies and researchers.

As a **Tier 2** suggestion, EPA should reconsider its carcinogenic MOA determination less the assessment be considered by many in the regulatory and scientific communities as markedly inconsistent with the latest scientific MOA weight of evidence evaluations by WHO, Health Canada, FSCJ and others. Additionally, moving forward in MOA analysis more generally, if a positive genotoxicity hazard finding in nontarget tissues nevertheless deemed relevant and sufficient by EPA (i.e., the assumption that genotoxic hazard = biological plausibility/possibility = demonstrated mutagenic MOA in target tissues) cannot be scientifically outweighed by results from MOA-focused studies on the relevant target tissues, in the species where tumors were observed, and at relevant doses known to be sufficient to induce the MOA that induced the tumors/cancers, then it seems this would/could/should be known prior to the conduction of any new MOA research (e.g., that EPA cannot exclude a potential mutagenic risk despite any MOA study results). If this is indeed the case, then EPA should consider explicitly stating this in appropriately nuanced and caveated text revisions to the cancer guidelines (EPA 2005a) since positive genotoxicity hazard findings in nontarget tissues are not uncommon, and more clear and transparent guidance might save time (e.g., regulatory agency assessment timelines), effort, and other precious resources (e.g., funding and laboratory animal lives) since it would be known that the sufficient demonstration of a MOA other than mutagenicity could not be met by new MOA research past that point (**Tier 2** suggestion). Indeed, given that the mutagenic MOA conclusion was drawn at the end of the genotoxicity hazard assessment (p. 3-111, lines 3-4), it is unclear if past that point any data could "demonstrate that a mutagenic MOA could reliably be excluded," which is the apparent current EPA operational standard for demonstrating a mutagenic MOA (i.e., not being able to exclude all mutagenic risk, as alluded to in EPA's presentation on slide 11 of the March 29, 2023.

Additional comments are provided below:

Reasonable scientific guidance for establishing a mutagenic MOA (EPA 2007, TCEQ 2015) does not support a mutagenic MOA for carcinogenicity having been demonstrated, particularly for the oral route. To elaborate on previous comments, a mutagenic MOA framework proposed by EPA (EPA 2007) outlines a multi-step process for evaluating the data to judge whether or not the chemical has a mutagenic MOA for carcinogenicity. EPA (2007) emphasized that, "The determination that a chemical carcinogen is capable of producing mutation is not sufficient to conclude that it causes specific tumors by a mutagenic MOA or that mutation is the only key event in the pathway to tumor induction," and that "For a chemical to act by a mutagenic MOA, either the chemical or its direct metabolite is the agent inducing the mutations that initiate cancer." Consistent with EPA (2007) and TCEQ (2015) guidance, one panel member strongly agrees that demonstration of a chemical's ability to cause mutations (or genotoxic endpoints such as chromosomal aberrations or MN in tissues not demonstrated to exhibit Cr(VI)-induced carcinogenesis) is not tantamount to a demonstration that the carcinogenic MOA is mutagenicity. Such evidence itself is insufficient. Mutagenicity induced by the chemical/metabolite must be *the key event that initiates the carcinogenic process*. Such a conclusion is not supported by dose-

response concordance analyses of target tissue mutagenicity/genotoxicity and subsequent tumorigenesis/carcinogenesis in those target tissues, or temporal concordance analyses (the most basic of the Hill criteria) of target tissue mutagenicity/genotoxicity with target tissue tumorigenesis/carcinogenesis,[18] to help demonstrate a biological relationship between target tissue mutagenicity (or genotoxicity) and subsequent tumorigenesis/carcinogenesis. For example, the most that Section 3.2.3.3 seeks to achieve seems to simply be showing that exposure to Cr(VI) is associated with some form(s) of genotoxicity in tissues (e.g., nasal or buccal cells, peripheral blood lymphocytes) that are not the target tissues for the dose-response assessment made basis for the draft SFo (duodenum tissues in the mouse). This section (and the *integration of genotoxicity evidence* section, p. 3-106) seems to treat any demonstration of genotoxicity in nontarget tissues, in human populations exposed to Cr(VI) through inhalation, as indicative of a mutagenic MOA for carcinogenesis in the small intestines of mice exposed orally. However, this type of information is wholly insufficient to conclude that Cr(VI) causes the mouse duodenal tumors (being used as surrogate data for humans) made basis for the draft SFo by a mutagenic MOA as it does nothing to demonstrate that Cr(VI) induces mutations that initiate these cancers (EPA 2007, TCEQ 2015). The draft contains no analyses such as the ones referenced above to help address this significant issue, although the importance of conducting such analyses for supporting a mutagenic MOA is recognized.[19] While EPA indicates that biomarkers found in the occupationally exposed (genotoxic effects in nasal or buccal cells, peripheral blood lymphocytes) have been shown to be positively associated with an increased risk of cancer in humans (p. 3-106), a leap of faith would be required to then assume/conclude that mutagenicity is the MOA operating to produce the critical tumors/cancers of concern used for dose-response analysis for application to the human population (in the small intestine of mice exposed to Cr(VI) through drinking water). Additionally, the strongest evidence as cited in the draft was obtained through the least relevant exposure scenarios (i.p. injection, in vitro). A mutagenic MOA determination is not supported as EPA presents no evidence, such as the analyses mentioned above (e.g., dose-response/temporal concordance), that demonstrates (or even strongly suggests) that the genotoxic effects discussed initiate the mouse small intestine tumors at issue for the draft SFo.[20] By contrast, the cytotoxicity-induced regenerative hyperplasia MOA is supported by evaluations of dose-response concordance and temporal concordance (e.g., see Figure 5 of Thompson *et al.* 2018 and Table 9 of Haney 2015c).

For consistency (i.e., to prevent what might be characterized as a double standard), EPA's deemphasis of genotoxicity results in nontarget tissue (applied by EPA in some cases) should be

[18] For example, TCEQ (2015) guidance states, "Lastly, a key issue is whether the observed dose-response relationships of the initial mutagenic events correspond with the dose-response relationship for tumors. Therefore, if possible, a comparison of the dose-response-temporal relationships between the occurrence of tumors and mutagenic/genotoxic effects known to be caused by the chemical (and perhaps even known to be present in the tumors) would be beneficial."

[19] EPA states, "…evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA" (p. 3-139, lines 26-28).

[20] Consistent with this and as mentioned above, the available data have led the World Health Organization (WHO 2020), Health Canada (2016), the Food Safety Commission of Japan (FSCJ 2019), the Texas Commission on Environmental Quality (TCEQ 2016), and others to recently conclude that the weight of evidence supports a different, threshold MOA. Consequently, it is obvious that EPA's conclusion is far from "settled science", which should be acknowledged in the main assessment (Tier 1 necessary revision mentioned in the main text of the comments).

applied consistently across results (see Tier 2 suggestions below). Table 3-19 (pp. 3-95 to 3-100) contains the prioritized genotoxicity studies in animals exposed to Cr(VI) categorized by tissue type. In the GI section, "rat small intestine is not a tumor target tissue" appears in comments for the Thompson *et al.* (2017) study, having the effect of diminishing the relevance of the results. [However, as Cr(VI) in drinking water did induce oral cavity cancers in the rat, this study did include a target tissue for which associated results are relevant to the carcinogenic MOA weight of evidence.] Consistent with EPA's use of the quote, as a Tier 2 suggestion, EPA should add "not a tumor target tissue" in the comments for each study in the "other tissues" section and the "tests using Cr(VI) to induce genotoxicity" section of Table 3-19, as results from these studies (comprising most of the table) are also not in target tissues. Any genotoxic effects observed in these nontarget tissues are of unknown relevance to the induction of cancer by Cr(VI) in target tissues as such effects are not known to lead to cancer in the very tissues in which they were observed. In the *Genes Mutation* section (pp. 3-100 to 3-102), two of the most species-, target tissue-, exposure route-, and draft SFo-relevant studies cited appear to be Aoki *et al.* (2019) and O'Brien *et al.* (2013), notwithstanding some cited aspects of the studies that could have been designed better. Although discussion of Thompson *et al.* (2017, 2015c) in this section seems to diminish the relevance of the results by stating that "inclusion of rat duodenal tissues in this mutation assay provides little value to mechanistic interpretation given the small intestine is not a tumor target tissue in rats," this ignores that the study did include a target tissue relevant to carcinogenic MOA analysis as Cr(VI) in drinking water induced oral cavity cancers in the rat. Regardless, the panel member did agree with the point EPA is trying to make and so suggest (Tier 2 suggestion) EPA apply this reasoning across studies where applicable. Consequently, consistent with the cited EPA language, as a Tier 2 suggestion, EPA should add text similar to the following to the discussions of all genotoxicity studies considered in the MOA analysis not conducted on tumor target tissues for the tested species, "evaluation of the tissue(s) utilized in this study provides little value to mechanistic interpretation given that the tissue(s) are not a tumor target tissue in the species tested." This consideration appropriately recognizes that even positive genotoxicity results in nontarget tissues (e.g., peripheral blood, bone marrow, retinal pigment epithelium) provide little value to mechanistic interpretation, and this should be accounted for in the weight of evidence for the carcinogenic MOA (Tier 2 suggestion). Nontarget tissue results are low on the hierarchy of evidence for determining a mutagenic MOA (EPA 2007, TCEQ 2015). The little value of nontarget tissue data to mechanistic interpretation should be recognized by EPA for other such results discussed elsewhere in the draft IRIS assessment as well (e.g., p53 gene expression suppression in the stomach and colon of rats (p. 3-128, lines 6-9) is not evidence of a mechanistic role in target tissue carcinogenesis as these tissues did not develop tumors).

In vivo MOA studies should not be devalued and/or rated "low confidence" by EPA for not including the maximum tolerate dose (MTD) (Tier 1 necessary revision) as they are designed to inform the MOA operating at doses known to sufficiently induce the MOA to produce tumorigenicity/ carcinogenicity in laboratory animals as opposed to being designed to more generally screen for genotoxicity hazard even under the worst of conditions (i.e., dosing at the MTD). Page 3-93 (lines 13-18) indicates, "The motivation for selecting a dose range to specifically study the induction of mutagenic effects at the same dose levels (albeit with shorter exposure durations) that caused preneoplastic lesions and tumors in these animals (e.g., up to 31.1 mg/kg-d Cr(VI) in female mice) is understandable. However, a bioassay properly designed

to detect potential mutagenic effects from ingested Cr(VI), a known carcinogen and a mutagen via other routes of exposure, was not identified." As this text seems oddly specific, it appears written to diminish results from certain studies in Table 3-19 (pp. 3-95 to 3-100). However, when attempting to understand the specific MOA that caused carcinogenic effects in a particular study, it only makes sense for studies focused on MOA (as opposed to genotoxicity hazard more broadly) to use the same species, exposure route, and doses (or drinking water concentrations).[21] If an even higher dose such as the MTD was not included in the positive carcinogenicity study then whatever may occur mechanistically at such doses (e.g., the MTD) but not at lower doses is not required to initiate cancer and could even potentially represent a dose-dependent transition in MOA that is not relevant to the MOA at the lower doses inducing cancer in the carcinogenicity study or to humans exposed environmentally (e.g., through drinking water). Results obtained at the MTD but not lower doses could be argued to have unknown relevance to the MOA occurring at lower carcinogenic doses where disruption of normal homeostasis and toxicity just below that causing animal death is not occurring. Several authors have suggested that exposure to high doses such as the MTD may cause cytotoxicity, leading to increased carcinogenicity due to an increased opportunity for cancerous mutations to arise during regenerative cell proliferation (e.g., Gaylor 2005), but this cytotoxicity-driven process for generating mutations does not comport with a mutagenic MOA wherein the chemical (or its direct metabolite) is the agent that induces the mutations that initiate cancer (EPA 2007, TCEQ 2015). Any positive results only occurring at the MTD would not be proof of a mutagenic MOA and might even be interpreted to support a different MOA considering the following good guidance [*emphasis added*] from EPA (2007), "Dose-response data may also suggest that the chemical does not act by a mutagenic MOA. For example, *if mutations occur only above doses that produce cytotoxicity or other impaired cellular functions, the observed mutations may be determined to be secondary to the other toxic effects*. Similarly, since in vivo mutagenic activity would generally be expected at doses lower than those that result in tumors, *the absence of mutagenicity at doses lower than those that cause cancer may suggest that mutagenicity is a secondary effect and, therefore, may suggest an MOA other than a mutagenic MOA*." If [*emphasis added*] "the absence of mutagenicity at doses *lower* than those that cause cancer may suggest that mutagenicity is a secondary effect," then the absence of mutagenicity at doses *equal to and greater than* the lowest dose sufficient to cause cancer may certainly suggest that "mutagenicity is a secondary effect and, therefore, may suggest an MOA other than a mutagenic MOA.*"* This cited EPA (2007) mutagenic MOA guidance is consistent with recent genotoxicity guidance from the International Programme on Chemical Safety (IPCS 2020) that indicates responses generated only at highly toxic doses or highly cytotoxic concentrations should be interpreted with caution, and the presence or absence of a dose-/concentration-response relationship should be considered (p. 4-38 of IPCS 2020). Thus, it appears IPCS (2020) would urge caution in interpreting a positive response only at the MTD and not at lower doses such as the carcinogenic doses in the NTP rodent and Cr(VI) MOA studies (constituting an absence of dose-response), which were still quite high (assuming that the highest doses used in the NTP mouse study did not in fact approach the MTD). According to the guidance cited above (e.g., EPA 2007, IPCS 2020), EPA is faulting Cr(VI) MOA studies for not using a dose (i.e., the MTD) where any positive genotoxicity results should not even necessarily be considered supportive of a mutagenic MOA, given the negative results at doses known to be

[21] For example, TCEQ (2015) guidance indicates, "Within the overall WOE approach for determining the likelihood of a mutagenic MOA for carcinogenicity, emphasis should be on evidence of mutagenicity being the initiating event in target cells at relevant doses (environmentally or to the carcinogenicity study)."

sufficient to induce the MOA causing tumors/cancers in mouse target tissues, but rather (consistent with guidance) could reasonably be interpreted to suggest that the genotoxic effects were "secondary to the other toxic effects" and that the MOA is "other than a mutagenic MOA." For EPA to seemingly dismiss or diminish results from MOA studies that assess mutagenicity/genotoxicity utilizing the same oral doses that caused cancer in the same species as not informative as to the MOA that was operating at those cancer-causing doses is surprising and not advisable as such data are the most directly relevant data available to elucidate the culpable MOA for tumorigenesis/ carcinogenesis. Consistent with potentially addressing this concern, previously it was suggested (**Tier 2** suggestion) that EPA reconsider its carcinogenic MOA determination.

Getting back to EPA concerns about study design, in EPA's review of relevant studies (negative studies in target tissues in particular), one panel member argued that while use of the MTD is important in genotoxicity hazard assessment to answer the question… "Does Cr(VI) have the ability to be genotoxic even under the worst of conditions designed to maximize the potential for a positive response (i.e., worst-case dosing)?", it is not the most relevant question for the MOA analysis. This is because the reasoned, good guidance in EPA (2007) emphasized that [*emphasis added*], "*The determination that a chemical carcinogen is capable of producing mutation is not sufficient to conclude that it causes specific tumors by a mutagenic MOA* or that mutation is the only key event in the pathway to tumor induction." Rather, the more relevant question for MOA analysis in the context of a dose-response assessment and draft carcinogenic potency factor (e.g., SFo) is more along the lines of… "What MOA was operating in target tissues at the doses known to be sufficient to induce the MOA and produce carcinogenic effects in the key animal study?" Consequently, not utilizing the MTD is a fair criticism of a genotoxicity/mutagenicity study designed to more generally screen for genotoxicity hazard even under the worst of conditions, but should not necessarily be viewed as a significant limitation in the context of a MOA study given its somewhat different purpose/focus. This is even more apparent when considering that [*emphasis added*]… "For a chemical to act by a mutagenic MOA, either the chemical or its direct metabolite is the agent inducing *the mutations that initiate cancer*" (EPA 2007), meaning that the mutations must be tied to the initiation of the cancer(s) in question to demonstrate a mutagenic MOA. Any attempt to do so is best accomplished through studies on the target tissue(s) using the same species, exposure route and doses/concentrations that produced cancer(s) in the key animal study for which the analysis is attempting to establish the carcinogenic MOA. *Such MOA studies are better suited to provide the data needed for the types of EPA-recognized analyses that can help establish the MOA (e.g., dose-response concordance and temporal analyses)*.[22] These comments are consistent with those of Dr. Toby G. Rossman, a genotoxicity/mutagenicity expert peer reviewer who participated in the *Peer Review Workshop for EPA's draft Toxicological Review of Hexavalent Chromium*. Dr. Rossman states, "Standard genotoxicity assays were not designed to inform specific modes of tumor induction" (p. A-55, July 6, 2011, post-meeting comments). By contrast, the Cr(VI) MOA studies were, which is again consistent with comments by Dr. Rossman [*emphasis added*]: For mutagenesis to be a carcinogenic MOA… the mutations should be induced *in a concentration range with low toxicity* (preferably similar to concentrations seen in human exposures), and the *mutations* should be

[22] EPA states, "…evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA" (p. 3-139, lines 26-28).

induced *in the target tissues in animal experiments* and in humans... *these should be early events* (p. A-55, July 6, 2011, post-meeting comments). The Cr(VI) MOA studies clearly meet these study design considerations, including both carcinogenic and lower doses, examining the target tissues identified in animal experiments, and looking for genotoxic/mutagenic effects early on. Additional comments consistent with these were provided by Dr. Sam M. Cohen, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center, indicating that: (1) when there is a known target tissue (e.g., the mouse duodenum), the genotoxicity assessment is to be made at the doses used in the chronic bioassay showing carcinogenic activity; (2) it is inappropriate to use higher doses as the effect of genotoxicity at doses higher than the dose for carcinogenicity completely negates one of the fundamental standards for mode of action analysis; that is, concordance between the dose of precursor changes (key events) and the dose-response for the actual adverse outcome; and (3) thus, for genotoxicity to be relevant to the carcinogenic effect, genotoxicity must be shown at the same or lower doses than the tumorigenic response (p. 1 of EPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2022). One panel member agrees. As a Tier 1 necessary revision, EPA should withdrawal its criticism of MOA studies for not using the MTD (while retaining it for any studies conducted primarily to assess genotoxicity hazard) and not continue to devalue and/or rate "low confidence" the genotoxicity results obtained from such studies.

Lastly, it seems that EPA's MOA assessment may be inconsistent with the EPA cancer guidelines (EPA 2005a). EPA (2005) states [*emphasis added*]: (1) "The approach to dose-response assessment for a particular agent is based on the conclusion reached as to its potential mode(s) of action *for each tumor type*" (Section 1.3.4); and (2) "The approach for extrapolation below the observed data considers *the understanding of the agent's mode of action at each tumor site*" (Section 3.3.1). This language emphasizes the importance of tumor type- and site-specific MOA data in agency decision making on dose-response assessment and low-dose extrapolation approaches; that is, it emphasizes the importance of MOA data collected on the specific target tissues that develop tumors/cancers at a site. Indeed, the understanding of the "MOA at each tumor site" (number (2) above) is inherently a function of the MOA data available for each tumor site. By contrast, for their draft MOA analysis, EPA lumps together information for different tissues and tumor sites (Table 3-21, pp. 3-144 to 3-152). This appears inconsistent with EPA (2005) and problematic for other reasons as well. For example, while MN have been found in oral epithelial cells of humans exposed via inhalation, the relevance and applicability of this finding is certainly dubious for the MOA inducing tumors at another site (small intestine), in a different species (mice), and at high known carcinogenic oral Cr(VI) doses given the negative results for Cr(VI)-induced duodenal crypt MN in MOA studies (Thompson *et al.* 2013, 2015). However, EPA nevertheless "expects" such evidence "to be applicable to all exposure types and tumors" (p. 3-142, lines 31-32). Accordingly, it is not clear that EPA's approach is tumor type- and site-specific as specified in EPA (2005), or that it considers the understanding of MOA for each tumor site that is inherently best a function of the MOA data actually obtained for each tumor site.

The detailed comments from another panel member:

> *It appears that some of the data could be described more clearly and/or accurately. For example, in Section 3.2.3 Cancer, the draft assessment indicates*

that hyperplastic responses did not increase in severity with dose (e.g., p. 134, lines 13-14). However, Table 4 from Cullen et al. (2016) contains results of a more thorough reevaluation of duodenal histological slides from the NTP 2-year Cr(VI) drinking water study and indicates an increase in the severity (and frequency) of epithelial hyperplasia associated with higher doses in female and male mice, which is illustrated by average severity score by dose in Figure 4 of that study. One might expect that more moderate degrees of hyperplasia would be associated with a longer time to tumor, whereas for a mutagenic MOA tumor responses generally occur early in chronic studies (within 52 weeks; EPA 2007), which was not the case for Cr(VI) (e.g., adenomas at >451 days). EPA should revise their statements that hyperplastic responses did not increase in severity with dose (e.g., p. 3-141, lines 25-26) in light of results from Cullen et al.. (2016) (Tier 1 necessary revision).

Also, in regard to data being described more clearly and/or accurately, p. 3-121 (lines 24-27) indicates that Thompson *et al.*, (2015b; 2015a) used X-ray fluorescence microspectroscopy to examine the concentrations of chromium in the cells residing within mouse villi and crypts, but that all analyses were performed in the middle section of the duodenum, which may be a significant source of bias. This may not be accurate as brief review of Thompson *et al.*, (2015b) indicates that use of the "Swiss roll" technique (a histologic preparation method) allowed the entire length the duodenum to be examined (e.g., see results and discussion section and Figures 2, S1 and S2). It seems EPA should revisit this to ensure that the cited text is accurate (Tier 2 suggestion).

In regard to synthesizing information in evaluating regenerative hyperplasia as a plausible MOA, regenerative hyperplasia should not be expected to produce genetic changes that initiate an observable tumor/cancer by end of study in every case but rather that regenerative hyperplasia significantly increases the chance that such changes will arise (i.e., it should be viewed as a necessary key event but not one that is not necessarily sufficient alone as the genetic changes that initiate cancer must still arise by chance). This is a reasonable and biologically plausible explanation for why not every mouse with epithelial hyperplasia should be expected to have a resultant observable tumor at the end of study. [Relatedly, it seems that the precursor hyperplastic lesion should not always be expected to be present at the time the cancer is discovered as the precursor lesion is frequently absent having been overtaken by the malignancy (e.g., many human colon cancers no longer have evidence of the precursor adenomatous polyp being present) (p. 2 of comments by Dr. Sam M. Cohen, MEPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2022).] Moreover, when combined with a significantly lower incidence and severity of epithelial hyperplasia in the rat (e.g., Table 5 of Cullen *et al.* 2016), this seems a reasonable and biologically plausible explanation for "the presence of degenerative lesions and hyperplasia in the rat small intestine with no induction of tumors at this site" (p. 3-134, lines 14-15). Considering the probabilistic nature of carcinogenesis and that NTP (2008) apparently did not even observe epithelial hyperplasia in the rat (see Tables A4 and B4 of NTP 2008), it is not surprising that cytotoxicity and regeneration in the rat was not sufficient

quantitively to lead to a detectable tumor response in the number of animals tested.[23] The EPA should consider these reasonings.

Also, in regard to synthesizing information, the draft indicates in several places (pp. 3-56, 3-105, 3-132, 3-141) that there were no statistically significant changes in mitotic indices in tissue regions where increased crypt length, area, and number of crypt enterocytes were reported, which EPA cites as a fault in the underlying evidence for regenerative hyperplasia as a plausible MOA. In regard to Thompson *et al.* (2015b), the draft states (p. 3-105, lines 22-23) that the "top dose did not induce a change in mitotic indices in the crypts which was interpreted as a lack of cytotoxicity, indicating a lack of sensitivity." As another example, this same consideration is applied to O'Brien *et al.* (2013) on the same page (lines 2-6). Professor Dr. Sam M. Cohen, Department of Pathology and Microbiology, University of Nebraska Medical Center, provides important external expert comment on this subject (p. 1 of EPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2022). Dr. Cohen indicates [*emphasis added*] that, "The difficulty that the draft IRIS assessment has is that they have interpreted a lack of an increase in the rate of mitotic activity with a lack of an increase in mitotic activity. This is categorically inaccurate. The key feature of increased cell proliferation relevant to carcinogenesis is the number of DNA replications, not the rate. The duodenum normally has a very high proliferation rate, which may not be increased with a hyperplastic response, and for that matter may not be increased to much of an extent in the development of adenomas or adenocarcinomas. *The key is that the number of crypt stem cells has markedly increased when there is hyperplasia. That is, there is an expansion of the crypt size due to an increase in the number of crypt stem cells. Even if the rate is the same as the controls, this reflects an increase in mitotic activity*. In general, mitotic rate is not used as a measure of mitotic and proliferative activity in small intestinal assessments, but rather, an evaluation of the size of the crypt, either actually counting the number of cells in the crypt or more commonly, as an easier method, is to measure the length of the crypt. In either case, *with regard to chromium administration in the drinking water, there is clearly an increase in mitotic activity as reflected in the expansion of the crypt, which is an increase in the number of stem cells, and which represents an increase in mitotic activity*." The EPA should consider Dr. Cohen's comments (e.g., "there is clearly an increase in mitotic activity as reflected in the expansion of the crypt") as they suggest that the draft assessment should not necessarily consider the lack of statistically significant changes in mitotic indices as an inconsistency in synthesizing and evaluating the evidence for regenerative hyperplasia as a plausible MOA (Tier 2 suggestion).

Additionally, EPA mentions worker buccal epithelial cells numerous times and cites chromosomal aberrations in the buccal epithelial cells of exposed workers as evidence of a mutagenic MOA (pp. 3-138 to 3-139). While EPA indicates that these biomarkers have been shown to be positively associated with an increased risk of cancer in humans (p. 3-106, lines 27-28), their relevance to the carcinogenic MOA operating in the small intestine of mice in particular is highly questionable, especially given the MOA studies that have been conducted on the relevant target tissues, in the relevant mouse strain, at doses more than sufficient to induce the MOA that produced tumors/cancers. The speculative relevance of buccal cell findings to the

[23] My opinion is consistent with that of Dr. Sam M. Cohen, Havlik-Wall Professor of Oncology, University of Nebraska Medical Center (see p. 3 of comments by Dr. Cohen, EPA-HQ-ORD-2014-0313-0038_attachment_1; December 18, 2022).

MOA should also be considered offset by results of the transgenic rodent (TGR) assay in Big Blue® rats, which should be considered by EPA as conducted at sufficiently high concentrations to inform the potential relevance of mutagenic risk for rat oral tumors through the oral drinking water exposure route to a high dose (see comments on MTD under question 6a). Exposure to 180 ppm Cr(VI) did not increase mutant frequency in the oral cavity of Big Blue® rats, and these results are directly relevant to informing the MOA at issue that is operating in the very target tissues that form one of the bases of concern for GI tract tumors for which the most appropriate low-dose extrapolation approach must be determined. In this regard, EPA should consider results of the TGR assay in Big Blue® rats more relevant and telling (e.g., than worker buccal cells) as to the MOA operating in the target tissues at issue (Tier 2 suggestion).

The draft assessment (p. 3-122, lines 2-5) indicates that a robust response in gene expression changes was detected in crypts at ≥4.6 mg Cr(VI)/kg-day and in villi at all doses ≥0.024 mg Cr(VI)/kg-day after 7 and 90 day exposures, demonstrating that Cr(VI) does reach the crypts at these concentrations in drinking water (Chappell *et al.*. 2022). In terms of synthesis, these data are consistent with the villi being more much more sensitive to Cr(VI)-induced changes in gene expression and with the induction of cytotoxicity-induced regenerative hyperplasia as the carcinogenic MOA, particularly since Cr(VI) induces small intestine tumors in mice at doses well below the 4.6 mg/kg-day where Cr(VI) has been demonstrated to affect gene expression in crypts (possibly adaptive changes in response to the villous damage that begins to occur at lower doses; Table 4 of Cullen *et al.*. 2016). For example, the study authors point out that "overall, the gene set enrichment in the villus demonstrated a cellular stress and damage response," that "consistent with histological evidence for crypt proliferation, a significant, dose-dependent increase in genes that regulate mitotic cell cycle was prominent in the crypt" while there was minimal transcriptomic evidence of DNA damage response in the crypts. EPA should consider more clearly acknowledging the consistency of these results with other relevant MOA study results supporting induction of a cytotoxicity-induced regenerative hyperplasia MOA (Tier 2 suggestion).

The draft IRIS assessment also states (p. 1-133, lines 27-30) that although the focal hyperplasia could be a part of the proliferative continuum of lesions, progressing from diffuse hyperplasia to focal hyperplasia (preneoplastic), to adenoma (autonomous growth), to carcinoma (malignant neoplasia) originating from a common precursor cell type, this cannot be confirmed. However, given the relatively low incidence of focal hyperplasia compared to that of diffuse hyperplasia in conjunction with other results from the MOA analysis studies (e.g., very early induction of diffuse epithelial hyperplasia but not focal hyperplasia, target tissue genotoxicity results), "part of the proliferative continuum of lesions" appears to be best supported by the relevant target tissue data while the alternative (direct neoplastic effects; p. 3-133, lines 26-27) appears to be speculative. That is, it appears that the conclusion that best comports with the data directly relevant to the carcinogenic MOA operating in target tissues to produce tumors via the oral route is that cytotoxicity-induced regenerative hyperplasia begins early with exposure to Cr(VI) and persists throughout a lifetime of exposure, which involves persistently increased stem cell divisions (within the crypt) due to proliferative pressure, and with them an increased probability of genetic alterations within the crypt that can lead to transformation and increased tumorigenesis/ carcinogenesis within the small intestine (e.g., duodenum) as the observable outcome. While EPA states (p. 3-156, lines 6-7) that, "Importantly, it is unlikely that this MOA

is solely operational in the intestinal tumors observed by NTP after 2 years," the basis for this statement is unclear. For example, while MOA study data establish that cytotoxicity-induced regenerative hyperplasia occurs early during mouse Cr(VI) oral exposure to the drinking water concentrations/doses known to induce the MOA that induce these tumors, EPA has not shown that their assumed MOA (i.e., mutagenicity) is operational in these target tissues under carcinogenic study relevant exposure conditions. Relevant analyses that would help support the likelihood that a potential MOA such as mutagenicity is "operational in the intestinal tumors" would include dose-response and temporal concordance analyses between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue, but there are no such supporting analyses although EPA recognizes the importance of conducting such analyses for supporting a mutagenic MOA.[24] Accordingly, EPA's basis for judging the probability (i.e., likelihood) that their assumed MOA (i.e., mutagenicity), or another, is actually operating to produce tumors/cancers in the mouse study target tissues is unclear.[25] Either data demonstrating that another carcinogenic MOA (e.g., mutagenicity) is indeed "operational in the intestinal tumors observed" should be cited by EPA to support this assertion or this otherwise seemingly insufficiently supported assertion should be removed (Tier 2 suggestion).

The draft assessment further states that, "Changes produced by the initiator may be latent for weeks or months and are considered irreversible. The hyperplasia observed at the 2-year evaluation endpoint may, therefore, be a manifestation of intestinal responses to late clonal expansion following an early initiation" (p. 3-155, lines 34-36). As this appears unsupported by relevant data for Cr(VI) in the present case, it appears to be speculation and EPA should consider removing it from the assessment (Tier 2 suggestion). Diffuse epithelial hyperplasia has been shown to occur very early upon Cr(VI) exposure, in the absence of demonstrated genotoxicity (e.g., Table 10 of Haney 2015c), and persists and increases in frequency with longer exposure durations (e.g., Table 8 of Haney 2015c). Importantly, the NTP (2008) study authors themselves concluded these lesions to be consistent with regenerative hyperplasia secondary to previous epithelial cell injury. Moreover, for a mutagenic MOA, tumor responses generally occur early in chronic studies (within 52 weeks; EPA 2007).

The above comments from individual panel members are provided for informational purposes to the EPA. As noted above, the majority of the panel (12 of 14 members) agreed that the evidence that Cr(VI) causes cancer through a mutagenic mode of action was sufficiently supported in experimental systems and was relevant to humans.

Charge Question #6b

The panel agreed that the dose-response decisions were transparent and recognized that the choice of linear extrapolation to estimate risk was consistent with EPA policy. However, the panel noted that there were multiple toxicokinetic factors that could significantly affect the shape

[24] EPA states, "…evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA" (p. 3-139, lines 26-28).

[25] As EPA states (p. 3-139, line 10), "The evidence for a mutagenic MOA following oral exposures is less clear."

of the dose response curve in the low to ultra-low dose region. Several factors could potentially be involved:

1) Diffusion from the extracellular to intracellular space (across the mucous layer);
2) Extracellular reduction of Cr(VI) into Cr(III) prior to uptake by the cell; and,
3) Inhibition of Cr(VI) uptake into the cell by sulfate and/or phosphate anions (Alexander and Aaseth, 1995) which are present at much higher concentrations in the extracellular fluid.

While the majority of the panel members supported the recommendation for a further evaluation by the EPA whether there is a potentially sublinear target deposition of Cr(VI) at low doses, an individual panel member indicated there is evidence against severe sub-linearity or threshold in absorption of low-dose Cr(VI) as follows:

- A study by the NTP (Collins *et al*., 2010) found linear and supralinear dose-dependence in chromium tissue accumulation in mice and rats following drinking water exposure with several concentrations of Cr(VI).
- Donaldson and Barreras (1966) found that duodenal administration of 20 ng Na_2CrO_4 in human volunteers resulted in approximately 50% tissue absorption, arguing against a super-efficiency of extracellular detoxification for ultralow doses of Cr(VI) in the small intestine.
- In addition to providing the most convincing evidence for a key role of sulfate transporters in chromate uptake by cells, Alexander and Aaseth, 1995 also found that sulfate was a very inefficient competitive inhibitor of chromate uptake (Fig. 4: only ~50% chromate uptake inhibition by 1260-fold excess of sulfate). This inefficiency results from a dramatically lower Km (~1 μM) for chromate uptake versus Km for sulfate (~100 μM and higher for SCL13 sulfate cotransporters, Bergeron *et al*. 2013). Physiological concentrations of sulfate in cell culture media are also known to permit efficient uptake of Cr(VI) at low micromolar concentrations with a linear dose-dependence (e.g., DeLoughery *et al*. 2015). It is not clear that significant concentrations of sulfate are even present in the small intestinal fluid.
- The assumption that there are higher rates of extracellular reduction/detoxification of Cr(VI) at low doses is not supported by mechanistic studies. The reduction of chromate by ascorbate, the principal reducer of Cr(VI) in biological systems at neutral pH, is a first order reaction (O'Brien and Woodbridge, 1997). The fundamental property of first order reactions is that the half-life is independent of the concentration of the minor component (i.e., Cr(VI)).

Overall, relative to a mutagenic mode of action the panel as a whole recognized that under the EPA (2005) cancer risk assessment guidelines, the low-dose linear approach is the default approach both when the mode of action is unknown and when a mutagenic MOA has been established. However, the panel also noted that due to toxicokinetic and other factors, the EPA Carcinogen Risk Assessment Guidelines (EPA, 2005a) allow deviations from the linear extrapolation approach, and that a non-linear (threshold) approach is used when a threshold MOA is clearly established. It states, "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." Two of the panel members and several of the public commenters

noted that there are several mutagenic or genotoxic carcinogens where the EPA had concluded, based on mechanistic evidence, that linear low dose extrapolation was not appropriate. These included captan, folpet, ethylene glycol monobutyl ether and ortho-phenylphenol. They also noted that captan and folpet involved small intestinal tumors in mice with a cytotoxicity/regenerative hyperplasia MOA. (Additional discussion on these points can be found in this appendix, Charge Question 6b1). It is important to acknowledge that the panel did not review EPA's rationale for its choice of the dose-response modeling approach for these other carcinogens.

- As indicated above, two of the panel members stated that the evidence supports a MOA involving cytotoxicity/regenerative hyperplasia and as a result, linear extrapolation into the low dose region is not appropriate. A detailed case was made for use of a non-linear extrapolation approach including evidence for non-linearity in the absorption of the Cr(VI) dose in the target tissue. A more thorough discussion of these points can be found here, in Appendix B, Charge Question - 6b2.

An individual panel member provided the following detail comments regarding linear extrapolation:

One panel member had the following comments: While linear extrapolation in the low dose region is consistent with the EPA default approach when dealing with a chemical that acts through a mutagenic mode of action, Cr(VI) is a unique agent and linear extrapolation may not be the best approach. While Cr(VI) may more directly come in contact with the lungs, it most likely will come in contact with bronchoalveolar fluid initially and then after diffusing across bronchoalveolar fluid will come in contact with the alveolar cells. At very low Cr(VI) concentrations, diffusion should be slower. It would also seem likely that ascorbic acid and/or other reducing agents which are present in the bronchoalveolar fluid (Schock *et al.* 2001, 2003) would be able to reduce Cr(VI) before it would be taken up by the cell. Similarly, it is well recognized that detoxification of orally ingested Cr(VI) will occur in the GI tract prior to Cr(VI). At very low doses and diffusing across the extracellular mucus, one might expect that reduction would be highly efficient, and may exceed the 80-90% values indicated in the review and the public comments. Furthermore, as indicated previously, at the very low concentrations of Cr(VI), competitive inhibition of Cr(VI) uptake by phosphate and sulfate present in the extracellular fluids may occur significantly reducing uptake into the cell by the anion transporters. Also, as described previously, intracellular reduction in the intestine and the lungs may also be seen as both an activation and inactivation pathway, particularly at very low chromium exposures. This combination of factors leads me to recommend further evaluation of the dose-response for Cr(VI)-induced cancer in the low, very low or ultra-low dose region to determine whether it would deviate from linearity and exhibit a sublinear or threshold-type of response.

The dose response decision was transparent and has been effectively justified, although as indicated above a different approach may be warranted. The study selections seem well thought out with appropriate decisions made to derive the PODs. EPA has made strong case that Cr(VI) is mutagenic and, that by inhalation exposure at high doses, it causes cancer through a mutagenic

mode of action. The evidence is not so strong with regards to oral exposure but the EPA has proceeded with a decision that Cr(VI) causes cancer through a mutagenic mode of action. It has then used its default position to use linear extrapolation to estimate risks in the low dose region. As mentioned above, one panel member believes that an additional evaluation of its mode of action in the low to very low dose region is warranted which could influence dose-response decisions. In my opinion, a determination that an agent is mutagenic at high doses should not necessarily indicate that it would be mutagenic at low doses, particularly if there are metabolic, toxicokinetic or repair processes that provide evidence that a non-linear approach would be more appropriate. In addition to folpet and captan discussed in Eastmond, 2012, and mentioned in the public comments, one panel member provided two examples, recognizing that both act through somewhat different mechanisms. The EPA IRIS Program concluded that ethylene glycol monobutyl ether, a cancer-inducing agent that, at high doses induces oxidative damage, including oxidative DNA damage, did not pose a significant carcinogenic risk at low doses (EPA, 2010; Eastmond, 2012). A similar conclusion based on different metabolic considerations was reached in the EPA Office of Prevention, Pesticides and Toxic Substances' evaluation of ortho-phenylphenol, a fungicide and disinfectant that at high doses induces chromosomal damage in the target organ (EPA OPPTS, 2006; Balakrishnan *et al.* 2002, 2006, 2016).

Another individual panel member provided the following comments regarding linear extrapolation:

Since part of the justification for a low-dose linearity approach is EPA's belief that a mutagenic MOA is responsible for Cr(VI)-induced carcinogenesis, comments above (under question 6a) also apply here. In concurrence with my own opinion, Dr. Toby G. Rossman, a genotoxicity/mutagenicity expert who participated in the *Peer Review Workshop for EPA's draft Toxicological Review of Hexavalent Chromium*, concluded that "the evidence for a mutagenic MOA is weak" and "I do not think that a linear no threshold approach is valid" (pp. A-57 and A-59, July 6, 2011, post-meeting comments). In addition to those comments (under question 6a), Table 3-1 (p. 3-4) of the draft assessment indicates that: (1) a gastric PBPK model of the stomach was used to estimate the Cr(VI) dose escaping stomach reduction, the resulting adjusted daily dose of which was used as the basis for an "internal" dose metric (really an adjusted applied dose) for dose-response modeling; and (2) stomach PBPK modeling of reduction/transit is sufficient for use in dose-response modeling without incorporating uptake kinetics. In regard to the Cr(VI) dose escaping stomach reduction (number (1) above) in the mouse, Figure C-12(a) of the draft assessment (p. C-28) shows that this "internal" dose used by EPA is essentially linear from higher-to-lower oral doses:

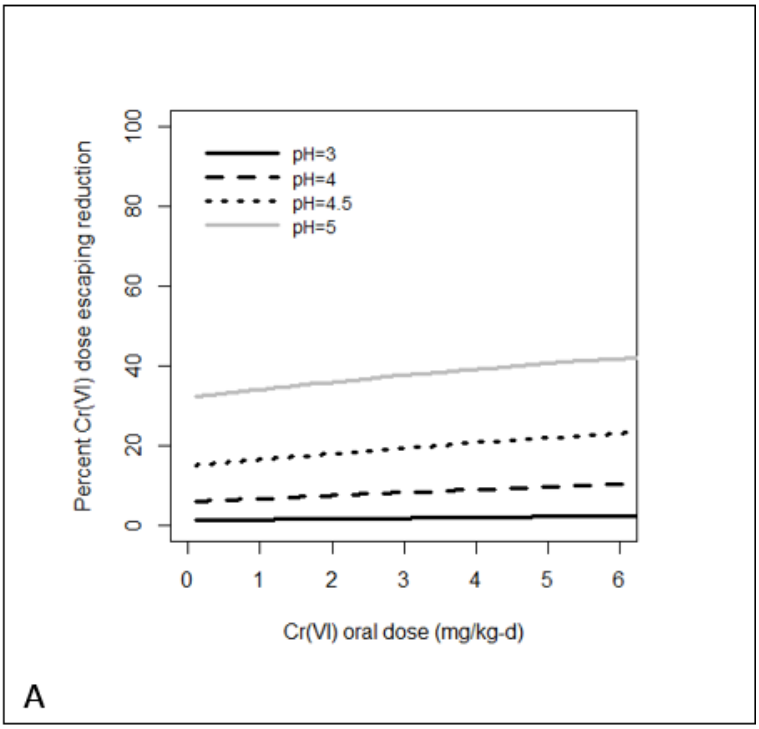


Figure C-12(a) of EPA's Draft Assessment

However, one panel member finds it difficult to support any such dose metric or extrapolation approach for estimating excess risks that does not reflect the apparent nonlinear dose-dependence of Cr(VI) absorption by target tissues in the mouse model being used as a surrogate for carcinogenicity in humans.

Based on rodent study target tissue data reported in Kirman *et al.* (2012), Haney (2015a,b,c) reports that target tissue Cr(VI) absorption, the actual/causal determinant of excess risk as all key events shown in Figure 3-16 of the draft occur following cellular uptake (also see the figures in Appendix A to these comments regarding the approximate relationship between mouse small intestine absorbed dose and the incidence of adenoma/carcinoma) and excess risk is proportional to target tissue absorbed dose,[26] is dose-dependent with progressively smaller fractions of the oral dose being absorbed by mouse target tissues as oral doses decrease below the draft SFo POD. For example, Figure 4 of Haney (2015a) shows dose fraction absorbed by target tissues (duodenum, jejunum, ileum) versus oral dose for drinking water concentrations of 0.3–60 mg SDD/L, which captures the draft SFo mouse POD dose and the oral dose at the MCL, and how the dose fraction absorbed at the draft SFo POD is higher than that at lower oral doses such as at the MCL.

[26] The aim of cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks (EPA 2005a), and EPA (1992) indicates that for toxicological equivalence in cross-species scaling, equivalent target tissue concentrations of the carcinogenic moiety may be assumed to give rise to equivalent degrees of impact at the cellular level and yield equal cancer risks (Section II.B.3).

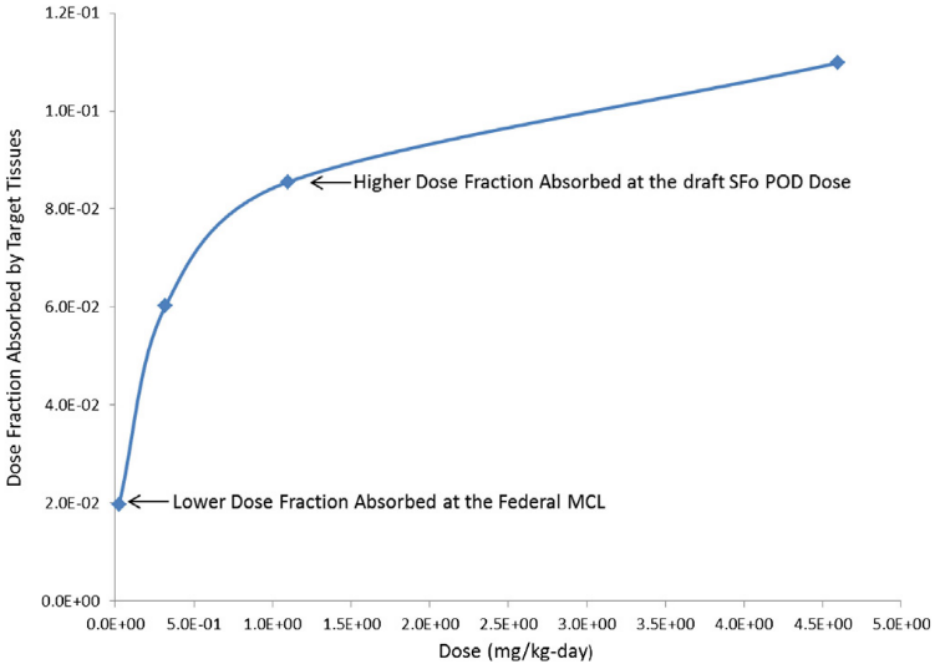


Fig. 4. Dose fraction absorbed versus dose.

Duodenum dose fraction absorbed results were very similar (see Table 9 of Haney 2015a) and the duodenum is where most tumors/cancers occurred. The following table (based on Haney 2015a) is provided to help discuss a duodenum-specific example of the implications of the nonlinearity of target tissue absorption below the draft SFo POD.

Overestimation of Duodenum Absorbed Dose by Assumed Linearity below the Draft SFo POD

Mouse Drinking Water Dose (mg Cr/kg-day)	**Notes**	**Duodenum Absorbed Dose** (mean added mg Cr/kg tissue)	**Linearity Predicted Duodenum Absorbed Dose Below Draft SFo POD** (mean added mg Cr/kg tissue) [c]	**Linearity Overpredicts Duodenum Absorbed Dose** (-fold over)
0		0	0	
0.008	1/3 federal MCL	0.009 [a]	0.052	**5.8**
0.024	at federal MCL	0.039 [b]	0.157	**4.0**
0.32		1.5 [b]	2.095	**1.4**
1.1	≈ draft SFo POD	7.2 [b]		

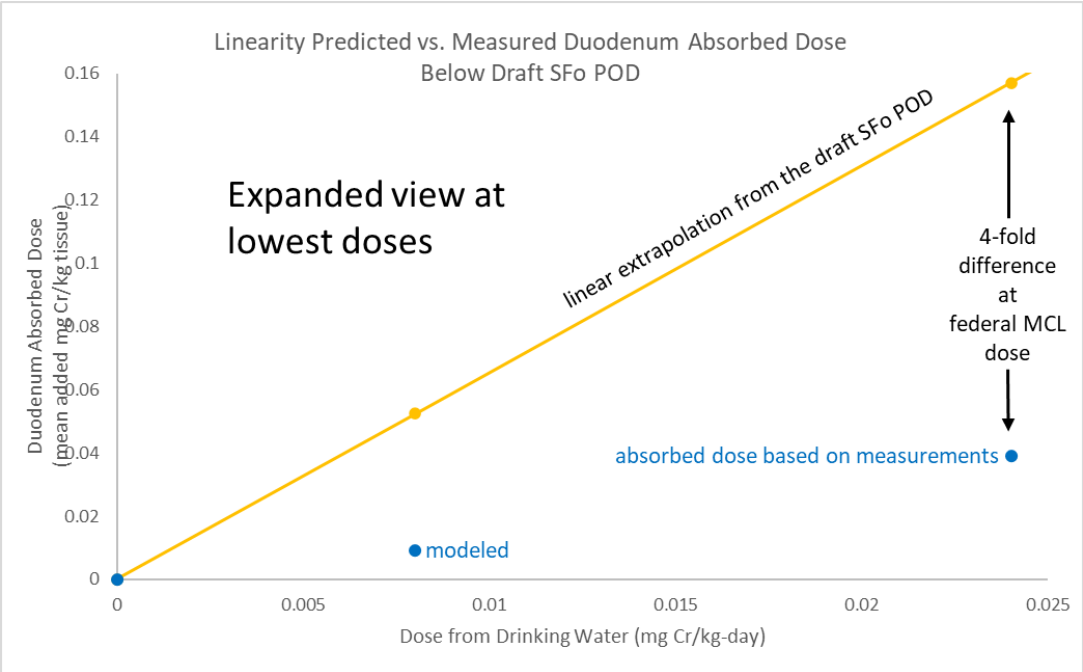
[a] Based on modeled tissue concentration for the mouse duodenum (Table 7 of Haney 2015a).

[b] Based on measured data (Table 2 of Haney 2015a).

[c] Predicted absorbed dose = (lower dose/POD dose) × duodenum absorbed dose at the POD.

At the approximate mouse SFo POD oral dose (≈1.1 mg/kg-d) the duodenum tissue absorbed dose was 7.2 added mg Cr/kg tissue. The mouse oral dose at the federal MCL was 0.024 mg/kg-

d, which is around 45.8-fold lower than the POD. With linear low-dose extrapolation, we would expect the duodenum tissue absorbed dose to also be around 46-fold lower (compared to duodenum tissue absorbed dose at the POD), giving rise to ≈1/46th the excess risk as would be estimated. However, the duodenum tissue absorbed dose was only 0.039 added mg Cr/kg tissue, which is a 184.6-fold lower target tissue absorbed dose. A 184.6-fold lower actual duodenum tissue absorbed dose/45.8-fold lower predicted (from linear extrapolation) gives rise to a 4-fold excess risk overestimation at the MCL when assuming low-dose linearity (i.e., target tissue dose is overestimated 4-fold as shown in the table above). Some figures are provided in Appendix B to these comments to help visualize the data in the table above, and the last figure is provided here for convenience.



Importantly, consideration of all three tissues (duodenum, jejunum, ileum) results in a similar overestimation at the MCL; a 4.3-fold overestimation (see Table 9 of Haney 2015a). EPA's "internal" dose metric (i.e., Cr(VI) dose escaping stomach reduction) does not reflect this nonlinearity in actual target tissue absorption, most specifically below the mouse SFo POD dose (≈1.1 mg/kg-d) from which excess risk is being extrapolated to lower doses (again, see EPA's Figure C-12(a) above).

It is also important to recognize that target tissue absorbed dose overestimation, and therefore excess risk overestimation, becomes progressively higher as oral doses progressively decrease from the mouse SFo POD, which again, is due to a progressively lower dose fraction being absorbed by target tissues as oral doses decrease below the POD (Figure 4 above; Haney 2015a,b,c).[27] The implication is that dose-dependent nonlinear target tissue absorption for Cr(VI)

[27] What then would be the risk overestimation at the average Cr(VI) drinking water concentration, which is perhaps over 100-fold lower than the MCL (based on data from the Third Unregulated Contaminant Monitoring Rule or UCMR3)?

at oral doses below the POD is inconsistent with linear low-dose extrapolation from the POD. That is, target tissue absorption of Cr(VI), and by corollary excess risk, does not extrapolate linearly below the draft SFo POD in the mouse model being used as a surrogate for humans, but rather linear low-dose extrapolation results in an overestimation that increases as oral doses decrease towards more environmentally-relevant doses (see the table above). Arguments that support nonlinearity in toxicokinetics, namely stomach reduction kinetic nonlinearity in mice and humans, from higher doses (e.g., draft SFo POD) to lower doses that by corollary affect target tissue dose and support a commensurate nonlinearity in extrapolated excess risk are also provided in public comments.[28] EPA acknowledges that nonlinearity in dose-response can often result from toxicokinetics (p. 3-5, EPA 2005a). As the mouse is being used as a surrogate for the carcinogenic dose-response in humans, it appears that this higher-to-lower dose nonlinearity in target tissue absorption (i.e., dose fraction absorbed) and excess risk should be reflected in the extrapolation approach (and resulting excess risk estimates) for humans. The EPA should reconsider their low-dose extrapolation approach in light of Cr(VI) nonlinear toxicokinetics such as those described above (**Tier 1**-necessary revision).

As just discussed, target tissue absorption data in the mouse model being used as a dose-response surrogate for humans indicate that target tissue absorbed dose, and therefore excess risk, is not linear below the POD as is inherently assumed by linear low-dose extrapolation. Since mouse data are being used as a surrogate for humans and show dose-dependence in the fraction of Cr(VI) dose absorbed by target tissues that results in nonlinearity in absorbed dose below the POD, any interspecies extrapolation that does not reflective this nonlinearity in target tissue absorbed dose (and thereby excess risk) may be viewed as inconsistent with the underlying surrogate mouse data, resulting in inaccurate excess risk estimates (overestimated risks in this case, even putting the MOA comments under question 6a aside). While modeling absorption by target tissues may have associated uncertainties (e.g., Table C-10, p. C-18), which are inevitably present in multiple areas of dose-response assessment, absorbed target tissue dose should generally be viewed as a better and more desirable dose metric for dose-response modeling as it is truly the internal dose most proximally, in fact causally, related to the tumorigenesis/carcinogenesis observed in the NTP rodent (e.g., mouse) studies. As stated in comments under question 4, EPA should reconsider their dose metric (Tier 1 necessary revision).[29] Since the mouse dose-response is being used as a surrogate for humans, target tissue absorption in the mouse could be modeled and include doses below the mouse draft SFo POD (e.g., Haney 2015a,b,c using data from Kirman *et al.* 2012) to characterize the nonlinearity in target tissue absorbed dose below the POD, and then that nonlinear relationship (or multiple doses that capture it) could be extrapolated to humans to reflect the nonlinearity in target tissue absorption that is apparent in the mouse below the POD and important in accurately

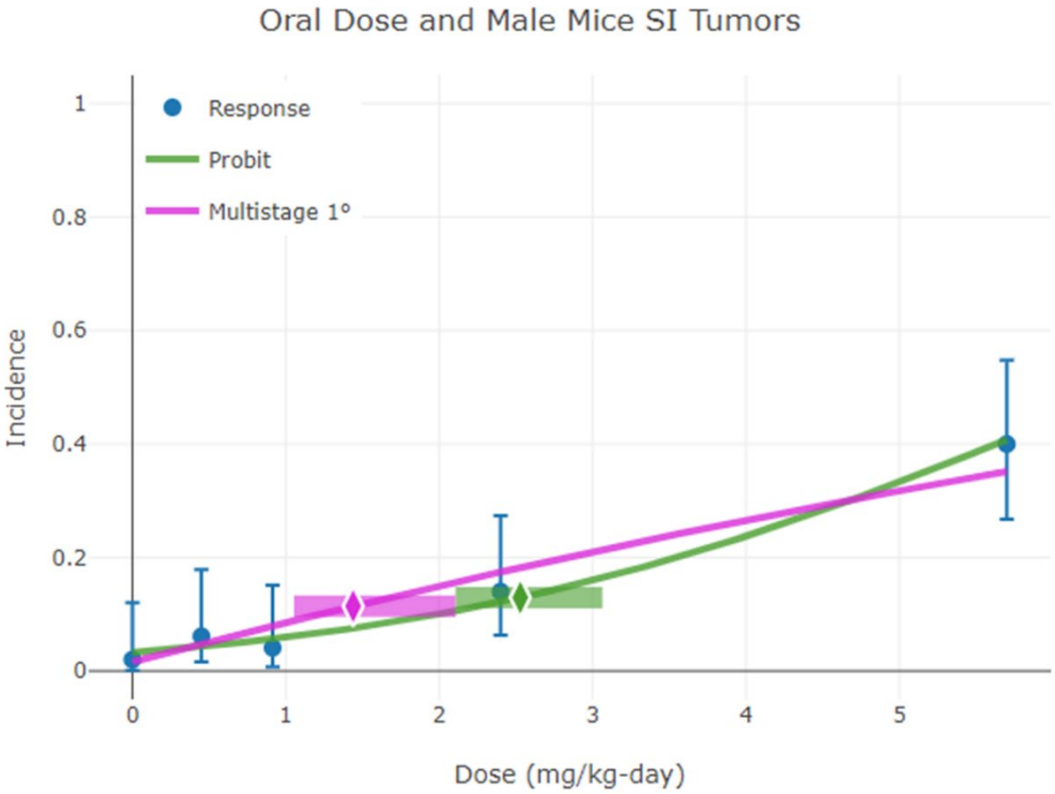
[28] For example, Figure 9 in the public comments by ToxStrategies (December 19, 2022; EPA-HQ-ORD-2014-0313-0045_attachment_1) shows that a clear dose-dependent transition in stomach reduction kinetics exists in mice and humans from higher doses (i.e., draft SFo POD) to lower doses, strong pharmacokinetic evidence supporting a non-linear dose-response.

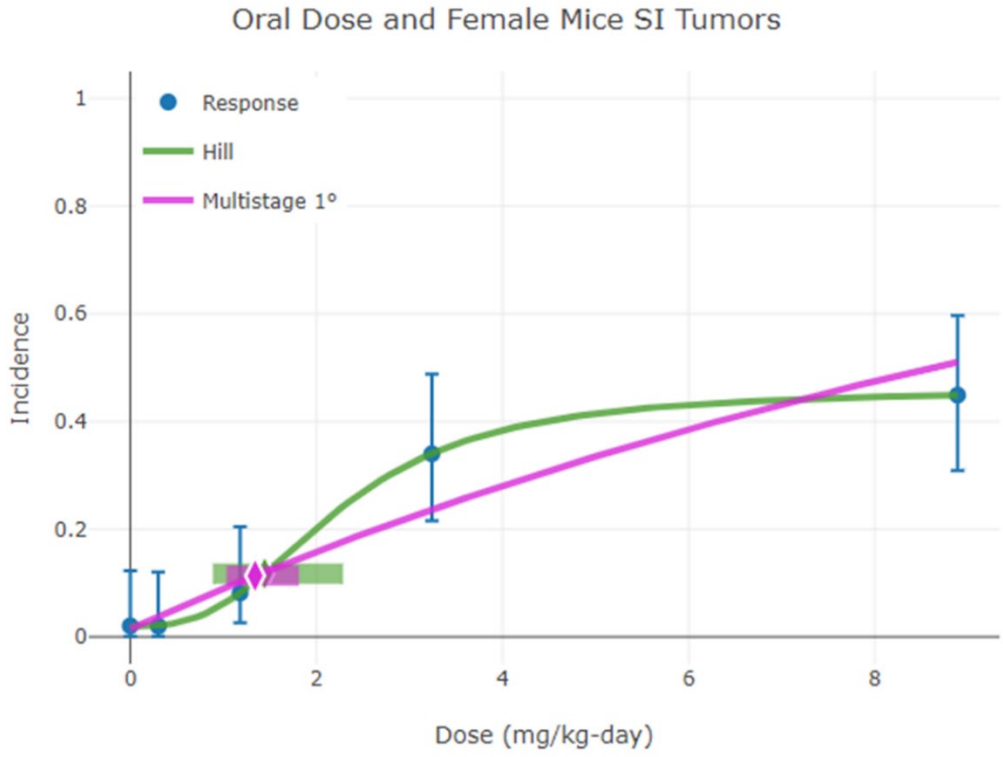
[29] For example, in comments under question 4, using target tissue absorbed dose as the dose metric for female mouse tumors, EPA's software suggested/draft assessment selected model (multistage degree 1) has an appreciably lower AIC (184.577) than the same model with the draft assessment dose metric (in mg/kg-d; 187.131). In fact, using target tissue absorbed dose as the dose metric, the majority of the BMD models have lower AIC values than the AIC for the model selected by EPA (187.131) using the draft assessment dose metric (mg/kg-d; see summary results in Appendix C).

extrapolating excess risk at lower doses. Use of a single point estimate (e.g., draft SFo POD) for low-dose extrapolation is incapable of reflecting dose-dependency below the POD in the dose fraction absorbed by target tissues of the mouse model being used as a dose-response surrogate for humans. For risk estimates to be considered scientifically defensible, nonlinearities in toxicokinetics such as (but not limited to) nonlinearity in target tissue absorption need to be appropriately accounted/adjusted for in the method EPA ultimately uses for estimates of excess risk (Tier 1 necessary revision). All this being said, given all that is known about Cr(VI) toxicokinetics and that EPA has PBPK modelers, one member noted that it should not fall on the SAB or others to have to demonstrate through analyses such as these that EPA should attempt exploring the implications of nonlinearities in Cr(VI) toxicokinetics for selecting the most appropriate low dose extrapolation method (or dose-response model) (Tier 1 necessary revision).[30] This is a reasonable consideration for EPA to explore in order to produce a sufficiently scientifically diligent and rigorous assessment, even in the absence of the information and analyses contained within these comments.

Lastly, overall, the dose-response data themselves do not appear to provide strong support for linear low-dose extrapolation but rather visual inspection reveals that nonlinearity is apparent for excess risk with oral dose. Consistent with this visual observation, *EPA BMDS suggests nonlinear models as the best-fitting models to the tumor data with oral dose*. For example:

[30] As examples, EPA acknowledges that nonlinearity in dose-response can often result from toxicokinetics (p. 3-5, EPA 2005a), Figure 9 in the public comments by ToxStrategies (December 19, 2022; EPA-HQ-ORD-2014-0313-0045_attachment_1) shows that a clear dose-dependent transition in stomach reduction kinetics exists in mice and humans from higher doses (i.e., draft SFo POD) to lower doses (strong pharmacokinetic evidence supporting a non-linear dose-response), and studies published by Haney (2015a,b,c) address the implications of nonlinearities in Cr(VI) toxicokinetics for linear low dose extrapolation.


Oral Dose and Male Mice SI Tumors
Response
Probit
Multistage 1°
Incidence
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Dose (mg/kg-day)


Oral Dose and Female Mice SI Tumors
Response
Hill
Multistage 1°
Incidence
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
Dose (mg/kg-day)

These best-fitting models extrapolate from the mouse draft SFo POD (≈1.1 mg/kg-day) to lower oral doses in a nonlinear way, so to impose linear low-dose extrapolation is not only contrary to the apparent toxicokinetics (as discussed above), but is also inconsistent with the shape of the actual dose-response data as modeled and confirmed visually. Indeed, EPA acknowledges that nonlinearity in dose-response can often result from toxicokinetics (p. 3-5, EPA 2005a). Figure 1 of Hartwig *et al.* (2020; no changes made other than to shorten the caption) provides a similar cancer dose-response curve where nonlinearity is apparent (note that the incidence axis only goes to 60%).

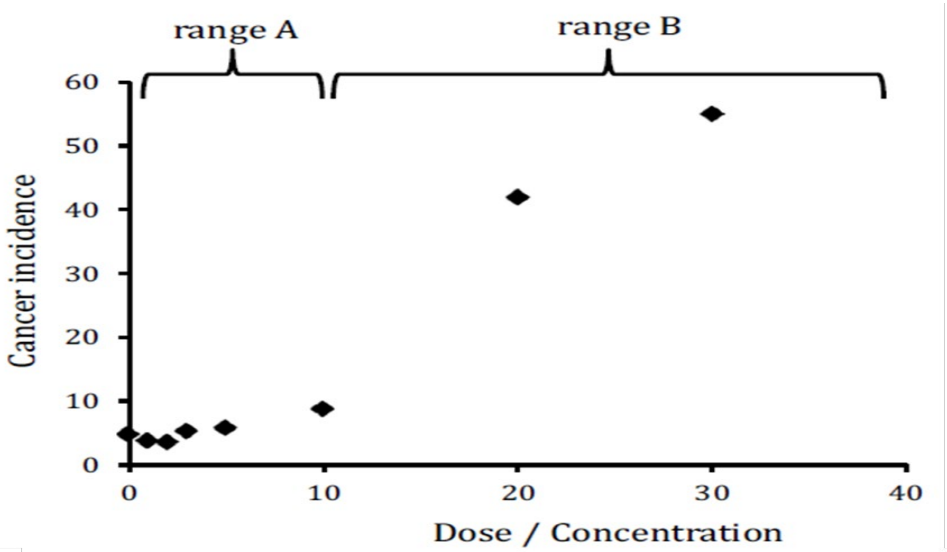


Fig. 1 Schematic graph of an apparently non-linear dose–response as observed in many cancer risk studies.

The study authors indicate that if a dose in the high dose range "B" (above where transitioning to a steeper slope has begun in the figure) is used for risk assessment by linear extrapolation, the cancer risk in the low-dose range "A" is likely to be overestimated. The figures above illustrate this overestimation in this particular case, where even in the observable range the linear models overestimate risk, with toxicokinetic considerations pointing to more drastic risk overestimations with progressively smaller oral dose fractions being absorbed as oral doses decrease to even lower levels (e.g., at the MCL, 1/3 MCL) below the draft SFo POD (as discussed above). While conservatism is supportive of the policy decision to default to linear low-dose extrapolation in the absence of scientific data to the contrary (e.g., pp. 1-19 to 1-20 of EPA 2005a), it should not be considered a defense, particularly a scientific one, in the present case where such data apparently exist (i.e., scientific data to the contrary, for toxicokinetics if not for MOA). The assessment should rely on analyses of data rather than general defaults. In contrast to reliance on defaults when data are relatively sparse, the EPA cancer guidelines (EPA 2005a) indicate that when more data are readily available, a critical analysis of all of the available data can be used as the starting point of the assessment (p. 1-6 of EPA 2005a), which in the present case includes those data supporting the nonlinear extrapolation of excess risk from higher doses (e.g., mouse SFo POD) to lower doses (e.g., toxicokinetic data such as (but not limited to) dose-dependency in the dose fraction absorbed by target tissues, the tumor dose-response data themselves and their best-fitting models). This is a Tier 1 necessary revision.

Comments regarding application of an age-dependent adjustment factor:

EPA should clarify the practical significance of an ADAF-adjusted SFo (Tier 2 suggestion). Briefly, EPA (2005b) supplemental guidance states that, "It is important to emphasize that these adjustments are combined with corresponding age-specific estimates of exposure to assess cancer risk," and states that EPA could provide sample calculations.

The panel generally supported the application of an age-dependent adjustment factor (ADAF) which accounts for increased early life susceptibility to carcinogens that have a mutagenic MOA, to both the oral cancer slope factor and the inhalation unit risk factor. As stated above, there is less mechanistic evidence for a mutagenic MOA for the oral route than for the inhalation route. One panel member reviewed EPA's rationale for applying/not applying ADAFs for the 20 other carcinogens (8 for which ADAFs were applied; 12 for which ADAFs were not applied) that IRIS has evaluated since the ADAF guidance (EPA (2005b) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens)* became available. The application of ADAFs to the oral slope factor for Cr(VI) was found to be consistent with EPA's decisions for these other carcinogens.

- Some panel members noted that if the additional toxicokinetic modeling indicated a sub-linear or threshold-like response in the very low dose region, then the application of ADAFs would not be appropriate.

- Additionally, the panel observed that age-dependent exposure to different media such as soil and water should be incorporated into the application of ADAFs when health-based criteria for such specific media are developed. Additional discussion on this point with an example from the IRIS Toxicological Review of Trichloroethylene can be found here, in Appendix B, Charge Question - 6b3.

A panel member who agreed with the application of ADAFs to account for increased risk of cancer in early life for both the oral and inhalation routes and had the following detailed comments: The application of ADAFs for the inhalation route is clearly appropriate for the inhalation route because a mutagenic MOA is definitively established.

<u>Application of ADAFs to derive slope factor of 0.5 (per mg/kg-day)</u>
(The comments below also apply to the oral slope factors for rat oral cavity tumors with and without ADAF adjustment presented on p. D-32, line 6.)

The discussion of adjustment of the slope factor with ADAFs should be revised to state that the slope factor of 0.3 (per mg/kg-day) applies to risks from less-than-lifetime exposures that begin in adulthood, such as occupational exposures. It should also be stated that ADAFs can be used to estimate risks from both lifetime exposures or from less-than-lifetime exposures during the early life period (**Tier 1:** Necessary Revision).

Importantly, EPA's application of the ADAFs to adjust the slope factor from 0.3 $(mg/kg\text{-}d)^{-1}$ to 0.5 $(mg/kg\text{-}d)^{-1}$ does not consider age-specific exposure assumptions, even though it is stated (p. 4-54, lines 19-21) that age-specific exposure assumptions were considered. As discussed in the EPA (2005) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*, adjustment of the slope factor to consider higher risks in early life involves combining ADAFs that account for higher susceptibility during each early-life period with age-specific exposure assumptions specific to the exposure medium and pathway of concern (e.g., ingestion of drinking water, incidental ingestion of soil, fish consumption). As such, the adjusted CSF will differ for different environmental media.

For examples of application of ADAFs combined with age-specific drinking water exposure factors, see Section 5.2.3.3.2 of the EPA (2011) *Toxicological Review of Trichloroethylene* at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0199tr/0199tr.pdf. The relevant table from p. 5-162 of this document is copied below.

Table 5-49. Sample calculation for total lifetime cancer risk based on the kidney cancer slope factor estimate, potential risk for NHL and liver cancer, and potential increased early-life susceptibility, assuming a constant lifetime exposure to 1 µg/L of TCE in drinking water

Column A	Column B	Column C	Column D	Column E	Column F	Column G	Column H	Column I	Column J	Column K	Column L
	Exposure scenario parameters				Dose-response assessment calculations						
Units:	L water/kg/d	mg/L water	yr	-	$(mg/kg/d)^{-1}$	-	-	$(mg/kg/d)^{-1}$	$(mg/kg/d)^{-1}$	-	-
Age group	Ingestion rate	Exposure concentration	Age group duration	Duration adjustment (Column D/ 70 yr)	Kidney cancer unadjusted lifetime slope factor (see Table 5-40)	Default ADAF	**Kidney cancer ADAF adjusted partial risk (Column B × Column C × Column E × Column F × Column G)**	Kidney cancer+NHL+ liver cancer unadjusted lifetime unit risk (see Section 5.2.2.3)	NHL+ liver cancer lifetime unit risk (Column I – Column F)	**NHL and liver cancer partial risk (Column B × Column C × Column E × Column J)**	**Total partial risk (Column H + Column K)**
Birth to <1 mo	0.235	0.001	0.083	0.0012	9.3×10^{-3}	10	$\mathbf{2.6 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.0 \times 10^{-8}}$	$\mathbf{3.6 \times 10^{-8}}$
1–<3 mo	0.228	0.001	0.167	0.0024	9.3×10^{-3}	10	$\mathbf{5.0 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.0 \times 10^{-8}}$	$\mathbf{7.0 \times 10^{-8}}$
3–<6 mo	0.148	0.001	0.250	0.0036	9.3×10^{-3}	10	$\mathbf{4.9 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.9 \times 10^{-8}}$	$\mathbf{6.9 \times 10^{-8}}$
6–<12 mo	0.112	0.001	0.500	0.0071	9.3×10^{-3}	10	$\mathbf{7.4 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.9 \times 10^{-8}}$	$\mathbf{1.0 \times 10^{-7}}$
1–<2 yrs	0.056	0.001	1.000	0.0143	9.3×10^{-3}	10	$\mathbf{7.4 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.9 \times 10^{-8}}$	$\mathbf{1.0 \times 10^{-7}}$
2–<3 yrs	0.052	0.001	1.000	0.0143	9.3×10^{-3}	3	$\mathbf{2.1 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.7 \times 10^{-8}}$	$\mathbf{4.8 \times 10^{-8}}$
3–<6 yrs	0.049	0.001	3.000	0.0429	9.3×10^{-3}	3	$\mathbf{5.9 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{7.7 \times 10^{-8}}$	$\mathbf{1.4 \times 10^{-7}}$
6–<11 yrs	0.035	0.001	5.000	0.0714	9.3×10^{-3}	3	$\mathbf{7.0 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{9.2 \times 10^{-8}}$	$\mathbf{1.6 \times 10^{-7}}$
11–<16 yrs	0.026	0.001	5.000	0.0714	9.3×10^{-3}	3	$\mathbf{5.2 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{6.8 \times 10^{-8}}$	$\mathbf{1.2 \times 10^{-7}}$
16–<18 yrs	0.024	0.001	2.000	0.0286	9.3×10^{-3}	1	$\mathbf{6.4 \times 10^{-9}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{2.8 \times 10^{-8}}$	$\mathbf{3.2 \times 10^{-8}}$
18–<21 yrs	0.029	0.001	3.000	0.0429	9.3×10^{-3}	1	$\mathbf{1.2 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{4.6 \times 10^{-8}}$	$\mathbf{5.7 \times 10^{-8}}$
21–<30 yrs	0.032	0.001	9.000	0.1286	9.3×10^{-3}	1	$\mathbf{3.8 \times 10^{-8}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{1.5 \times 10^{-7}}$	$\mathbf{1.9 \times 10^{-7}}$
30–70 yrs	0.032	0.001	40.000	0.5714	9.3×10^{-3}	1	$\mathbf{1.7 \times 10^{-7}}$	4.6×10^{-2}	3.7×10^{-2}	$\mathbf{6.7 \times 10^{-7}}$	$\mathbf{8.4 \times 10^{-7}}$
										Total unit risk:	$\mathbf{2.0 \times 10^{-6}}$

For another example, see the EPA Office of Water's use of this approach for 1,2,3-trichloropropane (presented on p. 4 of NJ DWQI, 2015, https://www.state.nj.us/dep/watersupply/pdf/123-tcp-appendixa.pdf).
Incidental soil ingestion is another exposure pathway of potential concern for Cr (VI) in New Jersey and other locations. As discussed for drinking water above, the Cr (VI) oral slope factor can be adjusted with ADAFs and age-specific soil ingestion assumptions (e.g., EPA, 2002; Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites,

https://semspub.epa.gov/work/HQ/175878.pdf) to estimate risks from early-life or lifetime exposure to Cr (VI) via incidental soil ingestion.

Slope factors for environmental media for which oral exposure is of concern for Cr (VI), including drinking water, soil, and any others, should be developed by application of ADAFs with consideration of age-specific exposure assumptions.

It is recommended that an explanation of the use of age-specific exposure assumptions in conjunction with ADAFs to develop adjusted slope factors for specific media (e.g., air, water) be added to the draft IRIS assessment. It would be helpful to readers if an example such as is provided on p. 5-62 of the EPA (2011) IRIS trichlorethylene assessment, shown above, be added. (**Tier 1:** Necessary Revision).

Charge Question #6c

All panel members agreed that the carcinogenicity data for the oral route of exposure support the conclusion that Cr(VI) is likely to be carcinogenic to the human GI tract. Detailed comments from individual panel members are provided below for informational purposes.

One panel member provided the following comments:

Yes, it appears that by and large, the available human and laboratory animal data on cancers of the GI tract are clearly and appropriately synthesized to describe the strengths and limitations (e.g., Section 3.2.3.2 of the draft). Section 3.3.3 indicates that under the 2005 Guidelines for Carcinogen Risk Assessment, Cr(VI) is "likely to be carcinogenic to humans" via the oral route of exposure based on: (1) a high confidence study in rodents showing a clear dose-response relationship between oral Cr(VI) exposure and incidence of GI tract tumors (NTP 2008); and (2) robust evidence that a mutagenic MOA has a key role in Cr(VI)-induced cancer via inhalation and oral exposures. Extensive comments on EPA's MOA conclusions and choice of laboratory animal model (mice) are provided elsewhere. Irrespective of those comments, the laboratory animal tumorigenesis/carcinogenesis drinking water study data suggest that assuming sufficiently high oral exposure over a sufficiently long duration (i.e., "given sufficient exposure conditions" as stated for other effects in the draft, however likely/unlikely those exposure conditions may be), Cr(VI) exposure has the ability to cause GI tract tumors/cancers in the general human population (including potentially susceptible subpopulations).[31] This being said, human data even in the occupationally exposed do not appear to this non-epidemiologist as particularly strong for demonstrating such exposure conditions/effects. Notably, the summary effect estimates from EPA's meta-analysis by cancer site in Table 3-14 (p. 3-70) are not

[31] To help prevent misinterpretation or an overly broad interpretation of this comment, note that my interpretation of "given sufficient exposure conditions" in this context means that primarily given the laboratory mouse evidence available for small intestine tumor/cancer effects due to oral exposure, oral exposure to Cr(VI) would be expected to produce excess risk for such effects in humans when dose and duration are sufficiently high and long to induce prolonged damage and regenerative hyperplasia in the GI tract/small intestine.

indicative of a strong association between Cr(VI) exposure and GI tract cancers in occupationally-exposed humans, even for the cancer with the strongest evidence, rectal cancer (see Figure C-20 below).[32]

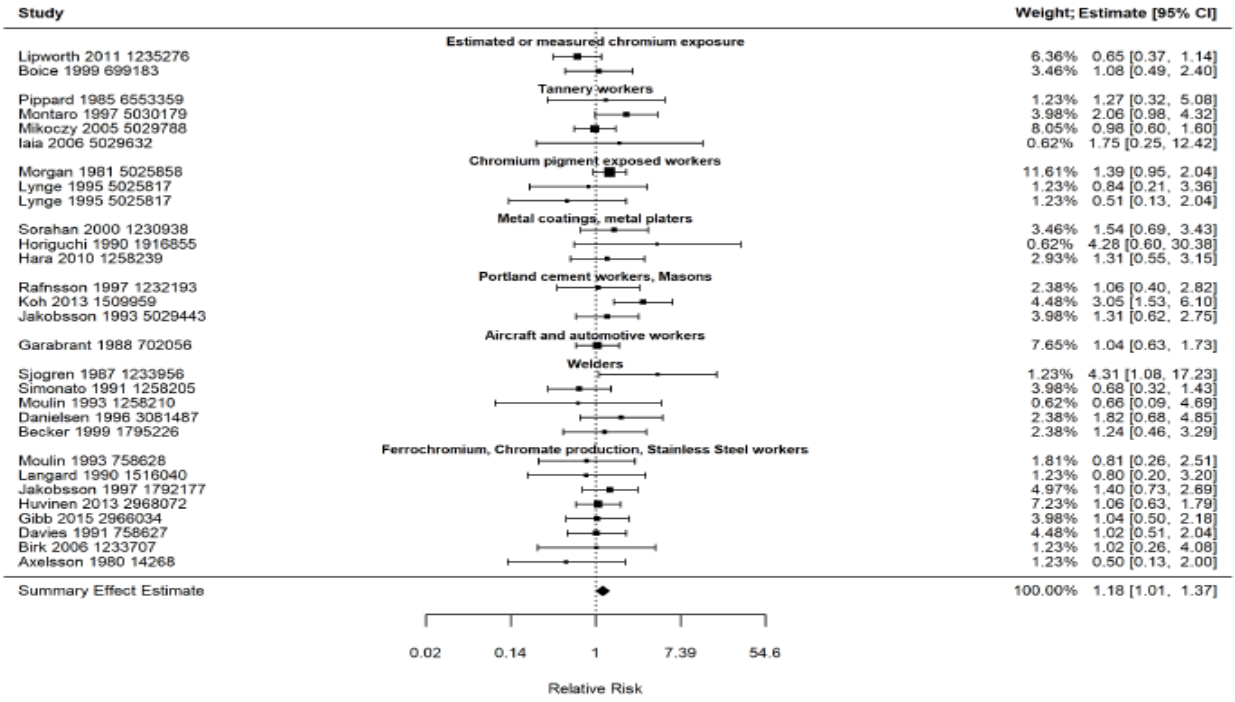


Figure C-20. Forest plot displaying summary measures for rectal cancer risk from studies reporting standardized mortality or incidence ratios.

In regard to potential SFo-based excess risk estimates for the human GI tract, as a mutagenic MOA has not been demonstrated by EPA (in my opinion) and is far from "settled science" considering that EPA's conclusion is at odds with the conclusions from several other recent carcinogenic MOA evaluations for Cr(VI) via the oral route (e.g., WHO 2020, Health Canada 2016, FSCJ 2019, TCEQ 2016), any such estimates should be considered to represent only hypothetical or theoretical risks based on an undemonstrated MOA. Dose-dependencies in the fraction of oral dose absorbed by target tissues (i.e., small intestine) and any other relevant toxicokinetic nonlinearities not sufficiently accounted for when extrapolating target tissue dose and excess risk from high-to-low doses (e.g., Figure 9 in EPA-HQ-ORD-2014-0313-0045_attachment_1 may be relevant, it better depicts the nonlinearities in human and rodent Cr(VI) reduction from higher doses such as the draft SFo POD to lower doses such as that at the MCL compared to Figure C-9(a) of the draft assessment) would further diminish the reliability and accuracy of such risk estimates (see comments under question 6b).

[32] EPA's meta-analysis reports RR/ORs of 1.01-1.43 with only rectum cancer (1.18 [1.01, 1.37]) having a lower bound of the confidence interval > 1, while SMRs for rectal cancer from Gatto et al. (2010) and Deng et al. (2019) were not statistically significant (see Table 2 of Gatto et al. and Table 3 of Deng et al.).

Charge Question #6d

The majority of the panel agreed with the selection of the study, endpoint, approach, and POD used by the EPA to calculate low dose risks of Cr(VI); however, if through additional modeling, the EPA concludes that another approach is more scientifically supportable, then that approach should be used. The detailed comments of one panel member are provided below for informational purposes.

The SFo has not been fully scientifically justified in my opinion. Upstream of this SFo derivation based on adenomas and carcinomas in the small intestine of mice, EPA has not attempted to scientifically justify whether the mouse or rat is more likely a better laboratory animal model for the same or similar effects in humans, or even if rodents are a good model for humans for gastrointestinal tract cancers more generally. EPA (2005) states (p. 3-24) that [*emphasis added*], "When multiple estimates can be developed, all datasets should be considered, and *a judgment made about how best to represent the human cancer risk*." Comparing the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals, Kararli (1995) concluded that while data indicate that no single animal can mimic the gastrointestinal characteristics of humans (i.e., human studies cannot be substituted by animals), the selection of the right animal model for a given purpose is possible. EPA should provide their basis for choosing the mouse dataset ("adenomas and carcinomas in the small intestine of male and female mice") as most representative of the overall dose-response in humans (Tier 1 necessary revision). For cancer effects from oral exposure, humans are assumed by EPA to have a dose-response (or POD at a minimum) similar to mice (i.e., mouse dose-response data, with dosimetric adjustments, are being used as the best laboratory animal surrogate data to represent dose-response in humans), but humans are assumed to have a dose-response like the rat for certain other effects evaluated in the draft assessment (e.g., liver effects, hematological effects). No scientific rationales are provided in the draft to support this data hopping from species-to-species for the specific species being considered most relevant for assessing the dose-response of a particular effect in humans. It is not scientifically robust to simply state, "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and this statement could be equally applied to negative findings in a given laboratory animal species. When not addressed scientifically, which is relatively common in my experience with few exceptions (e.g., male rat alpha 2u-globulin nephropathy), this can be a large and key area of uncertainty (e.g., where significant interspecies differences in sensitivity exist in the absence of data to inform identification of the most human-relevant laboratory animal species) that pertains directly to the meaningfulness of the resultant toxicity factor itself and for credibly informing risk management decisions.

The NRC has advised that proper characterization of uncertainty is essential in risk assessment as an assessment that omits or underestimates uncertainty can leave decisionmakers with a false sense of confidence in estimates of risk (NRC 1983,1994, 1996,2002). Use of an animal model as a surrogate for humans is an aspect of uncertainty that should be adequately addressed and characterized in an assessment (EPA 2005a). The uncertainty section (Section 4.3.5) recognizes this uncertainty. However, if EPA the finalizes the draft SFo, the assumption made by the public, risk managers and others as the SFo is utilized for purposes of risk assessment and as risk

management decisions are made will be that it is "settled science" that: (1) the same dose-response may be expected in humans (after appropriate dosimetric adjustments), and (2) the MOA is mutagenic, when in fact these issues are far from "settled science."[33] Consequently, similar to previous comments for other effects, as a Tier 1 necessary revision, in the interest of full transparency and clarity of presentation EPA should make abundantly clear that the choice of the most appropriate laboratory animal model for prediction of Cr(VI)-induced tumorigenesis/carcinogenesis in humans (if either is a human-predictive animal model) has not been scientifically established (rather species selection is based on policy)[34] and that reasonable scientists at other organizations, agencies and elsewhere continue to disagree on the carcinogenic MOA (i.e., the belief that the carcinogenic MOA is mutagenic is not based on "settled science"). Notably, although shown within the context of Table 4-15 (p. 4-57), the carcinogenic MOA is not considered worthy of discussion in the draft SFo uncertainty section of the main draft IRIS assessment (Section 4.3.5). This is a major area of uncertainty for the draft SFo and subject of continuing scientific debate, and exclusion from more detailed discussion in the uncertainty section of the main draft IRIS assessment (Section 4.3.5) is neither transparent nor acceptable (Tier 1 necessary revision for inclusion of such a discussion). *Other regulatory agencies and researchers have reasonably concluded that the carcinogenic MOA has not been demonstrated to be mutagenic and that the scientific weight of evidence best supports a different MOA*. For example, the recent assessment by the World Health Organization (WHO 2020) adopted a threshold MOA for Cr(VI)-induced carcinogenicity via oral exposure, indicating that weight-of-evidence analyses support a threshold MOA involving hyperplasia in the small intestine as a key precursor event to tumor development (p. 24 of WHO 2020).[35] Similarly, Health Canada (2016) evaluated the carcinogenic MOA weight of evidence and indicated that the carcinogenic MOA analysis supports hyperplasia as a key precursor event to tumor development and a threshold

[33] The notion of "settled science" in the area of environmental regulatory toxicology can be a perilous one. Our understanding of toxicology, chemical-specific effects and toxicokinetics, etc. is continuously expanding, pointing to the fact that existing science is incomplete and imperfect. A great number of scientific studies are published every year that continually increase our knowledge. This is why new dose-response assessments are conducted over time as new data emerge. Science is ongoing and our understanding is ever evolving. Consequently, the idea of "settled science" surrounding issues of ongoing scientific study and debate is often antithetical to science itself. It can also lead to misplaced priorities and diminished public health benefits based on science that may be shown in the near future to be flawed, or worse, can already be shown to be flawed. Both as scientists and a society we should be careful not to confuse "settled science" with science we have settled for (e.g., historical policy to avoid the critical scientific question of… What is the most human-relevant laboratory animal species for assessing the dose-response of this particular effect in humans?; definitive conclusions in the absence of sufficiently demonstrative evidence).

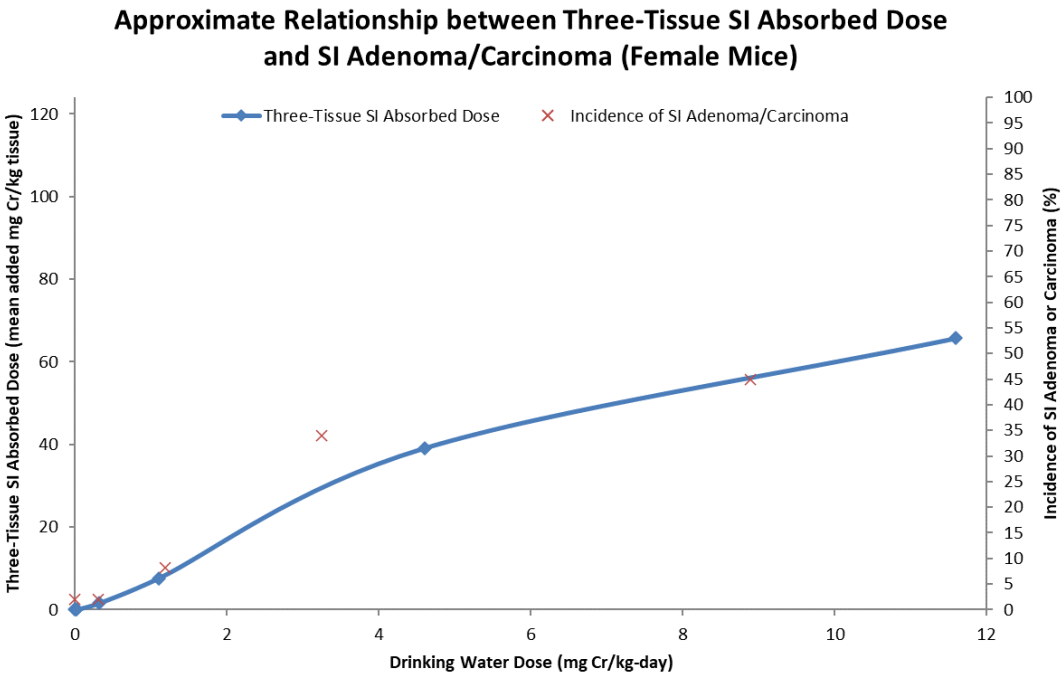
[34] While high confidence laboratory animal carcinogenicity studies provide good data to characterize the dose-response(s) for the species tested, the extent to which the selected species-specific data accurately predict carcinogenic effects in humans is unknown in the present case. This significant uncertainty applies to the high doses tested and even more so to the relatively low environmental exposures/doses of most interest for humans.

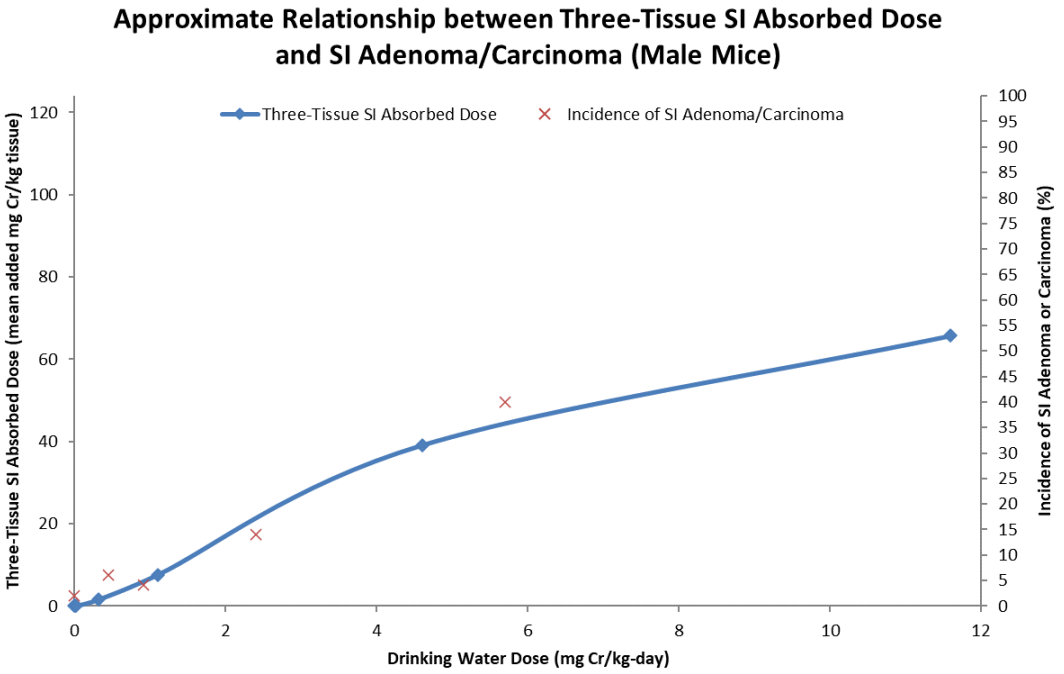
[35] For example, "Using the newer, high-quality data from chronic drinking-water carcinogenicity studies for Cr(III) and Cr(VI) (NTP, 2008a, b), and weight-of-evidence analyses supporting a threshold MOA (Health Canada, 2016), a GV of 50 µg/L remains valid (Moffat et al., 2018). The NTP (2008b) study allows a risk assessment of Cr(VI) in drinking-water that considers both cancer and noncancer effects, and provides evidence to support an MOA involving hyperplasia in the small intestine as a key precursor event to tumour development. Thus, a GV for Cr(VI) in drinking-water considering hyperplasia as the most sensitive end-point and precursor of tumour formation is protective of both cancer and noncancer effects. The current GV of 50 µg/L (total chromium) is therefore considered to be adequately protective of health and is retained, with the previously allocated 'provisional' status removed."

approach for the risk assessment for ingested Cr(VI), so diffuse hyperplasia of the small intestine was used by Health Canada as the most sensitive endpoint and precursor to tumor formation protective of both non-cancer and cancer effects (p. 59 of Health Canada 2016). The Food Safety Commission of Japan (FSCJ) has also adopted a threshold MOA for Cr(VI)-induced carcinogenicity via oral exposure, stating, "The mechanism of small intestinal tumors in mice is considered as follows: Continuous damage to mucosal epithelium in the small intestine by long-term exposure to Cr(VI) induces the hyperplasia in the crypt of small intestine, which would lead to the formation of tumor" and "Therefore, FSCJ chose the pre-cancerous lesion as the critical endpoint to specify TDI" (pp. 56 and 57 of FSCJ 2019). Like the carcinogenic MOA determinations by the WHO, Health Canada, and FSCJ, others have also evaluated the scientific evidence and reasonably concluded that the weight of evidence does not support a mutagenic MOA but rather supports a threshold MOA for Cr(VI)-induced tumorigenesis/carcinogenesis through the oral exposure route (e.g., Thompson *et al.* 2013, Haney 2015c/TCEQ2016). EPA's conclusion as to the carcinogenic MOA as well as subsequent modeling choices/assumptions (i.e., linear low-dose extrapolation) are at odds with these recent weight of evidence MOA determinations by other organizations, agencies and researchers (WHO, Health Canada, FSCJ, TCEQ and others). As a Tier 2 suggestion, EPA should reconsider their carcinogenic MOA determination in light of the latest scientific MOA data.

In regard to dose metric, target tissue absorbed dose is the most relevant and direct determinant of excess risk (e.g., all key events shown in Figure 3-16 of the draft occur following cellular uptake).[36] The figures below, provided in Appendix A with the underlying data, show the approximate relationship between mouse small intestine absorbed dose (mean added mg Cr/kg tissue) and the incidences of adenoma/carcinoma in the small intestine (SI) of female mice and male mice that EPA modeled (EPA 2021). There appears to be good agreement with the relevant data being from two mouse studies (i.e., Kirman *et al.* 2012 mouse PBPK study and NTP 2008).

[36] The aim of cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks (EPA 2005a), and EPA (1992) indicates that for toxicological equivalence in cross-species scaling, equivalent target tissue concentrations of the carcinogenic moiety may be assumed to give rise to equivalent degrees of impact at the cellular level and yield equal cancer risks (Section II.B.3).

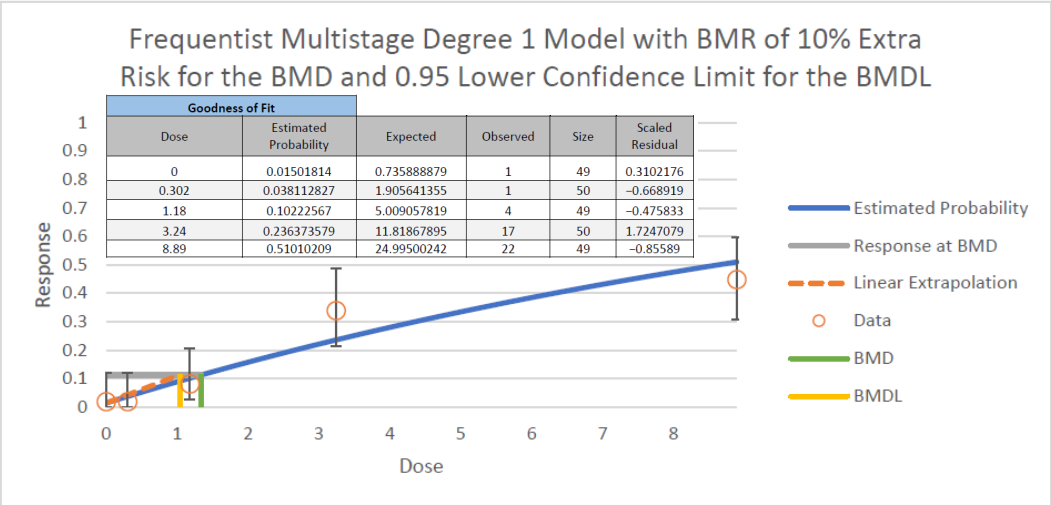




Based on the consideration of target tissue absorbed dose being the more direct determinant of excess risk, EPA may find that target tissue (e.g., three-tissue, duodenum, or duodenum + jejunum) absorbed dose provides a better fit to the data compared to, for instance, the following EPA BMD example from the model log file (p. 93 of EPA 2021). Target tissue absorption by the duodenum, jejunum, and ileum (or a subset) could be modeled to estimate the dose absorbed by each target tissue at the NTP (2008) study doses. A better fit to the adenoma/carcinoma incidence data, despite the potential uncertainties in Table C-10 (p. C-18), would increase

confidence in the use of target tissue absorbed dose (e.g., mean added mg Cr/kg tissue based on data from the Kirman *et al.* 2012 mouse PBPK study) as the preferred dose metric.

Analysis Name: Small intestine tumors (female mice) (NTP, 2008)



However, this is aside from a critical issue, namely that target tissue absorption appears to be dose-dependent and nonlinear (e.g., Haney 2015a,b,c). "Note that without evidence to the contrary, the human relevance of animal findings is assumed" (p. 1-17, lines 37-38), and dose-dependent nonlinear target tissue absorption is inconsistent with linear low-dose extrapolation conducted by EPA. The results in Table 9 of Haney (2015a), utilizing mouse tissue data from Kirman *et al.* (2012), show that the oral dose fraction absorbed by target tissues at the POD dose used in EPA's assessment for the draft SFo calculation ($BMDL_{10}$ values ≈1-1.1 mg/kg-day; Table ES-5) is approximately four times higher than that at the MCL and about six times higher than that predicted at one-third of the MCL. Figure 4 of Haney (2015a) shows dose fraction absorbed by target tissues (duodenum, jejunum, ileum) versus oral dose for drinking water concentrations of 0.3–60 mg SDD/L, which captures the draft SFo mouse POD dose and the oral dose at the MCL, and how the dose fraction absorbed at the draft SFo POD is higher than that at lower oral doses such as at the MCL. Duodenum-specific dose fraction absorbed results were very similar (see Table 9 of Haney 2015a) and a duodenum-specific example is provided in comments below.

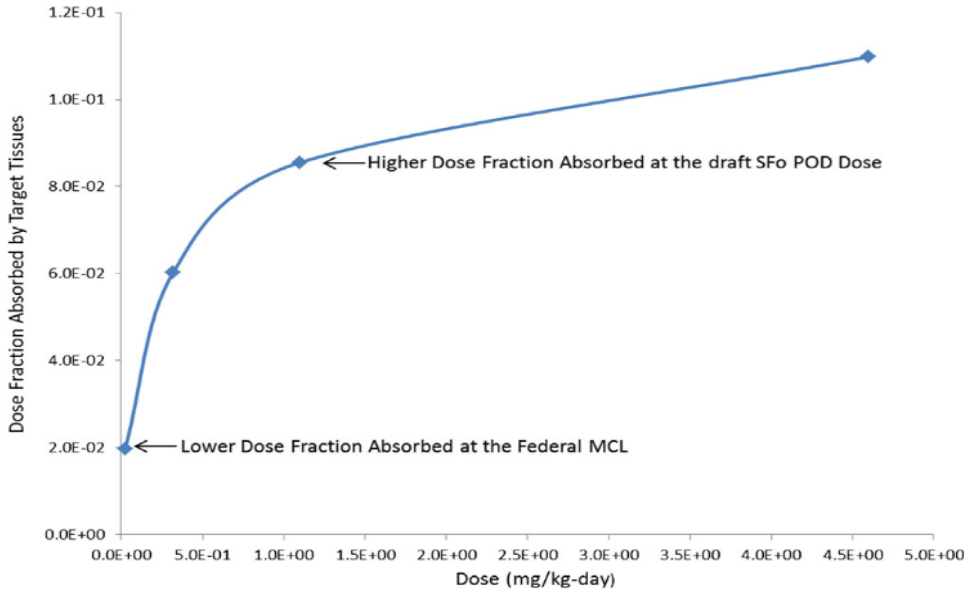


Fig. 4. Dose fraction absorbed versus dose.

Knowing this it becomes clear that target tissue absorbed dose, as the dose metric determinant of excess risk, is dose-dependent and nonlinear with oral dose, which violates the inherent underlying assumption of a SFo and its use for the calculation of risk; namely that target tissue dose is linear with oral dose such that, for example, one-tenth a given oral dose results in one-tenth the target tissue absorbed dose and thus one-tenth the excess risk. For this to be true, the slope of the line/data points in Figure 4 above would have to be (or at least approximate) zero with the line appearing horizontal.

The following table (based on Haney 2015a) is provided to help discuss an example of the implications of nonlinear target tissue absorption below the draft SFo POD by the duodenum specifically.

Overestimation of Duodenum Absorbed Dose by Assumed Linearity below the Draft SFo POD

Mouse Drinking Water Dose (mg Cr/kg-day)	**Notes**	**Duodenum Absorbed Dose** (mean added mg Cr/kg tissue)	**Linearity Predicted Duodenum Absorbed Dose Below Draft SFo POD** (mean added mg Cr/kg tissue) [c]	**Linearity Overpredicts Duodenum Absorbed Dose** (-fold over)
0		0	0	
0.008	1/3 federal MCL	0.009 [a]	0.052	**5.8**
0.024	at federal MCL	0.039 [b]	0.157	**4.0**
0.32		1.5 [b]	2.095	**1.4**
1.1	≈ draft SFo POD	7.2 [b]		

[a] Based on modeled tissue concentration for the mouse duodenum (Table 7 of Haney 2015a).

[b] Based on measured data (Table 2 of Haney 2015a).

[c] Predicted absorbed dose = (lower dose/POD dose) × duodenum absorbed dose at the POD.

At the approximate mouse SFo POD oral dose (≈1.1 mg/kg-d) the duodenum tissue absorbed dose was 7.2 added mg Cr/kg tissue. The mouse oral dose at the MCL was 0.024 mg/kg-d, which is around 45.8-fold lower than the POD. With linear low-dose extrapolation, we would expect the target tissue absorbed dose to also be around 46-fold lower (compared to target tissue absorbed dose at the POD), giving rise to ≈1/46th the excess risk as would be estimated. However, the duodenum tissue absorbed dose was 0.039 added mg Cr/kg tissue, which is a 184.6-fold lower target tissue absorbed dose. A 184.6-fold lower actual target tissue absorbed dose/45.8-fold lower predicted (from linear extrapolation) gives rise to a 4-fold risk overestimation at the MCL when assuming low-dose linearity (i.e., corresponding to the magnitude of target tissue absorbed dose overestimation). Figures are provided in Appendix B to these comments to help visualize this. Consideration of all three tissues (duodenum, jejunum, ileum) results in a similar overestimation at the MCL (i.e., a 4.3-fold overestimation; see Table 9 of Haney 2015a). Furthermore, it is important to recognize that risk overestimation becomes progressively higher as oral doses progressively decrease from the mouse SFo POD, which again, is due to a progressively lower oral dose fraction being absorbed by the duodenum target tissue as oral doses decrease (see Figure 4 above; Haney 2015a,b,c).[37] The implication is that dose-dependent nonlinear target tissue absorption for Cr(VI) is inconsistent with linear low-dose extrapolation conducted by EPA. As a Tier 1 necessary revision, EPA should reconsider this approach. All this being said, given that the dose actually absorbed by target tissues is the more proximate causal determinant of toxicity such as carcinogenic excess risk (e.g., all key events shown in Figure 3-16 of the draft occur following cellular uptake),[38] and given all that is known about Cr(VI) toxicokinetics and that EPA has PBPK modelers, one panel member noted that it should not fall on the SAB or others to have to demonstrate through analyses such as these that EPA should attempt utilizing target tissue absorbed dose as a dose metric and exploring the implications of nonlinearities in Cr(VI) toxicokinetics for selecting the most appropriate dose-response model or low dose extrapolation method (Tier 1 necessary revisions).[39] These are reasonable considerations for EPA to explore in order to produce a sufficiently scientifically diligent and rigorous assessment even in the absence of the information and analyses contained within these comments.

Compounding these significant uncertainties related to MOA, animal model, and the apparent nonlinear dose-dependence of target tissue (i.e., small intestine) absorption, EPA acknowledges and outlines significant modeling and other uncertainties. See Section 4.3.5 and other EPA-referenced sections of the draft assessment. Recognizing and considering the magnitude and

[37] What then would be the risk overestimation at the average Cr(VI) drinking water concentration, which is perhaps over 100-fold lower than the MCL (based on data from the Third Unregulated Contaminant Monitoring Rule or UCMR3)?

[38] The aim of cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks (EPA 2005a), and EPA (1992) indicates that for toxicological equivalence in cross-species scaling, equivalent target tissue concentrations of the carcinogenic moiety may be assumed to give rise to equivalent degrees of impact at the cellular level and yield equal cancer risks (Section II.B.3).

[39] As examples, EPA acknowledges that nonlinearity in dose-response can often result from toxicokinetics (p. 3-5, EPA 2005a), Figure 9 in the public comments by ToxStrategies (December 19, 2022; EPA-HQ-ORD-2014-0313-0045_attachment_1) shows that a clear dose-dependent transition in stomach reduction kinetics exists in mice and humans from higher doses (i.e., draft SFo POD) to lower doses (strong pharmacokinetic evidence supporting a non-linear dose-response), and studies published by Haney (2015a,b,c) address the implications of nonlinearities in Cr(VI) toxicokinetics for linear low dose extrapolation.

implications of these significant uncertainties, one panel member remarked that confidence in the draft SFo appears quite low indeed.

Another panel member stated that the MOA is a major area of uncertainty for the draft oral slope factor and a subject of ongoing scientific debate; therefore, a more transparent and detailed discussion should be included in the uncertainty section of the main draft IRIS assessment (Section 4.3.5).

Charge Question #6e

The panel agreed that the available data for the IUR have been appropriately synthesized to describe the strengths and limitations and do support the conclusions presented. The comments of one panel member are provided below for informational purposes.

Section 4.4.2 (p. 4-67) indicates that a BMR of 1% extra risk was used to estimate a POD. However, there is no discussion in this section regarding whether the POD is within or near the range of the dose-response data… "near the lower end of the observed range, without significant extrapolation to lower doses" (EPA 2005a, p. 1-13). While Section 4.4.3.1 (p. 4-71) indicates that a "1% value is used because lung cancer is a severe adverse effect and 1% also represents a lung cancer response level that is near the low end of the observable range (U.S. EPA, 2012)… also consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012b)", the citations provided regard BMD modeling software and guidance and not cohort-specific information. Section 4.4.5.8 of the uncertainty section, however, indicates that PODs (i.e., LEC_{01} of 0.899 μg Cr(VI)/m^3 for the Cox analysis, 0.951 μg Cr(VI)/m^3 for the R&L analysis) were between the minimum exposure level (i.e., zero) and the 25th percentile from Table 2 of Gibb et al. (2015). While technically true, one member voiced that including exposure estimates of zero for unexposed workers in a cohort may not be what EPA (2005) envisions when evaluating whether a POD is "near the lower end of the observed range".[40] As a Tier 2 suggestion, EPA should attempt to use cohort-specific information for the exposed to confirm consistency with EPA (2005) that the POD at a BMR of 1% extra risk is "near the lower end of the observed range, without significant extrapolation to lower doses."

Section 4.4.3.2 (p. 4-73, lines 2-4) indicates that part of the reason that the Cox proportional hazards model was selected for the Cr(VI) IUR is that results from this type of model have been used as the basis for EPA IRIS IUR derivations for breast cancer (EPA 2016b) and lymphohematopoietic cancer (EPA 2016b). However, as results of the Cox proportional hazards model were rejected by EPA as the basis for these IURs in EPA (2016b), this reason should be removed (Tier 2 suggestion).

Last but not least, many of the comments under 6(a) above regarding a mutagenic MOA not having been demonstrated also apply here. Following what is a genotoxicity hazard assessment,

[40] The zero exposure estimate could have been for the worker(s) employed 0.003 years with 0.3 years of follow-up (Table 2 of Gibb et al. 2015).

just one part of a MOA analysis (TCEQ 2015, EPA 2005a),[41] EPA rather simply relies on genotoxicity as a plausible/possible carcinogenic MOA, stating, "In conclusion, there is consistent and coherent evidence that a mutagenic MOA for Cr(VI)-induced carcinogenesis is biologically plausible and relevant to humans" (similar statement on p. 3-130, lines 5-6). The primary basis for EPA's conclusion as to the carcinogenic MOA was simply a genotoxicity assessment... genotoxicity = biological plausibility (i.e., a possible MOA) = demonstrated mutagenic MOA. EPA presented evidence for Cr(VI)-induced genotoxicity, but no Cr(VI)-specific evidence for establishing a mutagenic MOA past that. This is true for the cancers made basis for the draft IUR, where EPA's MOA conclusion relies on two main considerations spread over six numbered statements[42]: (1) not all Cr(VI) being reduced extracellularly prior to absorption (particularly at high deposition sites where it can cause lung tumors); and (2) examples of Cr(VI) exhibiting genotoxicity in occupationally exposed humans (exfoliated nasal and buccal cells, peripheral blood, lung cell cultures) and laboratory animals (mouse lung). EPA has conducted no analyses that show or suggest causation or dose-response/temporal concordance between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue, although EPA recognizes the importance of conducting such analyses for supporting a mutagenic MOA.[43] Indeed, an important criterion in EPA (2005) is "similar dose-response relationships for tumor and mode of action-related effects" (Section 2.3.5.4 *Judging Data*) or "dose-response concordance" (p. 2-45), and a demonstration of temporality is the most basic of the Hill criteria (p. 2-45 of EPA 2005a). Based on the study data discussed, one member concluded that there are no Cr(VI)-specific data in the draft assessment that demonstrate a connection between genotoxicity in the target tissue and the initiation of tumors/cancers in the target tissue (contrary to the solid arrows in Figures 3-16 and 3-18 and the statement on p. 3-137 lines 22-23). Therefore, EPA must rely on assumption and speculation rather than such analyses in an attempt to draw a connection to explain the MOA operating in Cr(VI) target tissues.[44] EPA

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[41] Two key weight of evidence determinations are involved in applying the EPA (2007) framework. They generally concern the critical underlying questions of interest: (1) Does the carcinogen demonstrate mutagenic activity?; and (2) Is the carcinogen operating via a mutagenic MOA in the cancer target tissue? (TCEQ 2015, p. 162).

[42] EPA states, "Therefore, a mutagenic MOA for lung tumors is considered to be relevant to humans and sufficiently supported in laboratory animals after inhalation exposure, based on the following: 1) the evidence-based interpretation that some amount of inhaled Cr(VI) (at physiologically relevant doses) escapes detoxification and is taken up by target cells; 2) this uptake is expected to occur more readily in regions of the lung showing a high chromate deposition that correlate with sites of lung tumors in exposed workers; 3) demonstrations of increased chromosomal mutations in the exfoliated nasal and buccal cells and in the peripheral blood of occupationally exposed workers; 4) gene mutations in the mouse lung that increased with dose and time post-intratracheal instillation; 5) other genotoxic effects in the peripheral blood of exposed workers and in lung-derived cell cultures in vitro; and 6) mutagenicity of Cr(VI) when it reaches cells of various tissue types in vivo and in vitro." (pp. 3-138 to 3-139).

[43] EPA states, "…evidence of mutation in the tumor target tissue occurs earlier than the induction of tumors, in the same species, and at the same doses causing tumors supports a mutagenic MOA" (p. 3-139, lines 26-28).

[44] Table 3-122 ("gene and chromosomal mutation" row on p. 3-116) states "Bulky Cr-DNA lesions lead to replication fork stalling and DNA double-strand breaks, which can become fixed mutations if not efficiently repaired or targeted for cell death by apoptosis. Some of these mutation may confer a growth advantage, leading to a clonal outgrowth of the mutated cells and tumorigenesis, a process that is more likely to occur in rapidly proliferating cells." This is speculative and is not tantamount to data allowing a solid arrow to tumors in Figure 3-16 (p. 3-113); that is, it is unknown if this is the process initiating Cr(VI)-induced tumors, so as

concentrated on genotoxicity hazard assessment to draw the MOA conclusion at the end of the genotoxicity hazard assessment (p. 3-111, lines 3-4); assembling data to most simply show that Cr(VI) has been shown to be capable of inducing genotoxicity. To then assume that genotoxicity must therefore be the carcinogenic MOA operating in target tissues requires a leap of faith due to both a lack of supporting data in the present case as well as it being contrary to good guidance on evaluating the potential for a mutagenic MOA (e.g., EPA 2007, TCEQ 2015). The lack of data (e.g., dose-response/temporal concordance analyses) that demonstrate a connection between genotoxicity in the target tissue and the initiation of tumors/ cancers in the target tissue precludes any demonstration or conclusion that the carcinogenic MOA is a mutagenic one. To assume that demonstration of genotoxicity = biological plausibility/possibility = demonstrated mutagenic MOA in target tissues is too low of a bar (EPA 2007, TCEQ 2015) as it is not a scientifically robust one. Consistent with previous comments above, EPA's mutagenic MOA conclusion as applied to both oral and inhalation routes is far from "settled science" (e.g., WHO 2020, Health Canada 2016, TCEQ 2016, FSCJ 2019, Proctor *et al.* 2014), which should be acknowledged in the assessment in interest of full transparency (Tier 1 necessary revision).

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such it is speculation, Figure 3-16 should not have solid arrows between these speculative events and the tumors. Speculating a mutagenic MOA is also revealed in important text such as [*emphasis added*], "…the ability of Cr(VI) to reach the crypts (where stem cells reside), *which could give rise to* cytotoxicity as well as fixed *mutations*…" (p. 3-121, lines 2-3).

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