December 19, 2022

Comments on the Draft IRIS Toxicological Review of Hexavalent Chromium

Comments submitted via regulations.gov to the docket ID EPA-HQ-ORD-2014-0313

These comments are submitted on behalf of the undersigned scientists. We declare collectively that we have no direct or indirect financial or fiduciary interest in the subject of these comments. The co-signers' institutional affiliations are included for identification purposes only and do not imply institutional endorsement or support unless indicated otherwise.

We appreciate the opportunity to provide written comments on the October 2022 External Review Draft *Toxicological Review of Hexavalent Chromium [Cr(VI)]* and *Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment*. These are important products of EPA's Integrated Risk Information System (IRIS) program, both because of the serious health risks posed by exposure to Cr(VI) and because this assessment is an important milestone in the implementation of systematic review methods by EPA. We believe this is the first major IRIS assessment for a "legacy" substance with a large evidence base in which systematic review methods have been applied from start to finish. The assessment includes all the required major components of a systematic review, including a protocol outlining the approach to evidence identification, evidence evaluation, data extraction, evidence synthesis and evidence integration. The IRIS program has made significant progress in implementing systematic review and the Cr(VI) is an impressive accomplishment in many respects. While these comments focus in large part on areas for improvement, we wish to reiterate that overall the Cr(VI) assessment represents an important step forward in systematic review for EPA and that IRIS program methods (with the necessary incorporation of NASEM recommendations on the draft Handbook) should serve as a model for systematic review in other Agency programs.

Our comments address the following main issues:

1. **The draft assessment was conducted following a protocol that clearly outlined the methods applied to the assessment in advance. The protocol is largely consistent with the 2020 draft *ORD Staff Handbook for Developing IRIS Assessments* and does not address the 2022 NASEM recommendations for improvements to IRIS systematic review procedures presented in the draft Handbook.**
    a. **The final protocol was not released before the assessment was conducted, and changes from the draft protocol are not clearly explained**
    b. **The protocol lacks clarity and consistency in study evaluation concepts regarding sensitivity and reporting quality**
    c. **The protocol does not incorporate financial conflict of interest into risk of bias assessment**
    d. **The protocol retains the practice of excluding studies rated to be "critically deficient" in a single domain, and of allowing studies rated as "low confidence" to be disregarded. Contrary**

to NASEM recommendations, numerous studies are excluded from the Cr(VI) assessment based solely on study quality ratings.

2. **Evidence synthesis and integration for non-cancer outcomes should be improved.**
   a. **The distinction between evidence synthesis and evidence integration should be clarified**
   b. **Evidence synthesis classifications appear to understate the strength of the evidence for some health outcomes.**

3. **Selection of studies for dose-response analysis and derivation of candidate RfDs is clear and well-justified. However, insufficient justification is provided for selection of GI tract effects rather than liver effects for the overall RfD.**

4. **EPA's conclusions regarding carcinogenicity of Cr(VI) by the oral route of exposure are well-supported.**

We appreciate the opportunity to provide input. Please do not hesitate to contact us with any questions regarding these comments.

Sincerely,

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DETAILED COMMENTS

1. **The draft assessment was conducted following a protocol that clearly outlined the methods applied to the assessment in advance. The protocol is largely consistent with the 2020 draft *ORD Staff Handbook for Developing IRIS Assessments* and does not address the 2022 NASEM recommendations for improvements to IRIS systematic review procedures presented in the draft Handbook.**

Development of the draft Cr(VI) assessment apparently took place in parallel with review and revision of the draft *ORD Staff Handbook for Developing IRIS Assessments: 2020 Version*. EPA initially published a draft Cr(VI) assessment protocol[1] for public comment in 2019, and published a revised protocol[2] October 2022 at the same time it released the draft assessment for peer review and public comment. In 2020, EPA released the draft *ORD Staff Handbook for Developing IRIS Assessments* for public comment and review by the National Academies of Sciences, Engineering, and Medicine (NASEM). In 2022, the NASEM issued its report on the draft Handbook, *Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version*. The NASEM committee stated that it is "is impressed and encouraged by the progress that the IRIS program has made to date"[3] and provided "recommendations that it believes are critical for improving the scientific rigor and clarity of the handbook."[4]

The revised Cr(VI) protocol incorporates revisions to address comments on the 2019 draft and, in the "Protocol History" section, provides a 16-item bullet-point list that briefly summarizes those revisions. Among the listed revisions to the Cr(VI) protocol in the 2022 version is "Revised methods and text to align with draft handbook."[5] Our review of the 2022 Cr(VI) protocol finds that it is largely consistent with the 2020 draft IRIS Handbook, and generally does not incorporate the 2021 NASEM recommendations for improvements in the IRIS program implementation of systematic review. It appears that the Cr(VI) assessment proceeded concurrent with the NASEM review rather than waiting for NASEM recommendations, which was a reasonable decision to maintain IRIS program productivity. However, it does mean that some concerning aspects of the draft Handbook methods continue to be employed in the draft Cr(VI) assessment.

[1] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials, 2019). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-18/155, 2019.

[2] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022.

[3] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, pages 1-2.

[4] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 3.

[5] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 126.

a. The final protocol was not released before the assessment was conducted, and changes from the draft protocol are not clearly explained

Pre-publication of a draft protocol, taking public comments on the protocol, publishing a revised protocol to address comments, and identifying the revisions are all important accomplishments in the implementation of systematic review in the IRIS program and are consistent with best practices in systematic review.[6,7,8] The list of protocol revisions, however, is quite terse and lacks clear identification and the changes made, specific sections of the protocol where the revisions can be found, and the rationale for revisions. Expanded identification and discussion of the protocol revisions would constitute a significant advance in transparency of the assessment. This expanded discussion of the revisions can still be added to the Cr(VI) protocol, and the IRIS program should adopt this as standard practice for all future assessments.

A related issue is that the revised protocol was published concurrent with release of the draft assessment. Best practices for systematic review include publication of a final protocol before the assessment is conducted, and IRIS should meet this standard for future assessments. (We note that the IRIS program did publish a revised protocol, after receiving public comments and prior to conducting assessments for five per- and polyfluoroalkyl substances (PFAS)[9], which we hope is an indication of future program practice.) Additional documentation regarding whether the protocol revisions were made prior to conducting the Cr(VI) review would be an important enhancement to transparency.

The NASEM recommended advance publication of the final IRIS systematic review protocols, with:

> revisions or refinements documented and the **final draft of the assessment protocol registered in advance of the detailed analysis of the hazard identification and dose-response stages of the assessment**. Any deviations from the planned methods described in the registered assessment protocol can be documented when the draft IRIS assessment is released for public comment and peer review.[10] (emphasis added)
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> **Recommendation 3.6**: EPA should create a time-stamped, read-only final version of each document that details the planned methods for an IRIS assessment prior to conducting the assessment. *[Tier 1]*[11]

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[6] National Academies of Sciences, Engineering, and Medicine. The Use of Systematic Review in EPA's Toxic Substances Control Act Risk Evaluations. Washington, DC: The National Academies Press, 2021.

[7] Institute of Medicine. *Finding What Works in Health Care: Standards for Systematic Reviews*. Washington, DC: The National Academies Press, 2011

[8] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022.

[9] U.S. EPA. Systematic Review Protocol for the PFAS IRIS Assessments (2021). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-19/050, 2019.

[10] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 41.

[11] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 49.

b. The protocol lacks clarity and consistency in study evaluation concepts regarding sensitivity and reporting quality

The approach to study quality evaluation for the Cr(VI) assessment includes consideration of risk of bias, sensitivity and reporting quality, which differs from other validated systematic review methods.[12,13] Figure 2 of the Cr(VI) protocol shows the study quality evaluation domains for epidemiology (list) and animal studies (list), which are unchanged from those shown in Figure 6-1 of the draft Handbook.

The NASEM review of the draft IRIS Handbook commented on the novelty of the IRIS approach and the study evaluation domains:

> While standard methods for systematic review would consider only risk of bias when evaluating individual studies (Higgins and Thomas, 2019), study evaluation in the handbook is intentionally broadened to include the additional study evaluation domains of "sensitivity" and "reporting quality." It is not standard practice to include the concepts of sensitivity or reporting quality as part of the evaluation of individual studies included in systematic reviews of human research.[14]

The NASEM further advised to EPA to reconsider and refine its concepts of sensitivity and reporting quality as they relate to study quality assessment and other steps in the systematic review process:

> **Finding**: The quality assessment item described as "sensitivity" covers important concepts, but is ambiguous and under-operationalized, as it covers aspects of internal validity, external validity, and statistical precision that overlap with other more commonly accepted features of systematic review that may be better assessed at other stages of the systematic review process.[15]
>
> **Recommendation 4.2**: EPA should evaluate whether aspects currently captured in the notion of "sensitivity" might be better described in the handbook with more established terminology (e.g., precision or generalizability) or better addressed at other points of the systematic review (e.g., risk of bias assessment or evaluation relative to PECO statement[s]). Otherwise, the handbook should provide a more concrete definition of "sensitivity" and a procedure for operationalizing its use in the study evaluation step. *[Tier 1]*[16]

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[12] National Toxicology Program Office of Health Assessment and Translation. Handbook for Conducting a Literature-Based Health Assessment Using OHAT Approach for Systematic Review and Evidence Integration. National Institute of Environmental Health Sciences; 2015.

[13] Woodruff TJ, Sutton P. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environmental Health Perspectives. 2014;122(10):1007-1014. doi:10.1289/ehp.1307175.

[14] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 52.

[15] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 61.

[16] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 61.

Finding: The use of reporting quality as a distinct quality assessment item for study evaluation is not standard for systematic reviews, and procedures for evaluating reporting quality are very different for the human epidemiological and animal toxicological studies. Reporting quality is included within other evaluation domains for human studies, with almost no specific guidance on how to incorporate reporting quality within these domains. This presents the possibility of downgrading a study quality rating without any specific evaluation criteria.[17]

Recommendation 4.3: The handbook should address the apparent difference in assessing reporting quality between the human epidemiological and animal toxicological studies by either (1) assessing reporting quality similarly in both types of studies or (2) providing an explicit rationale for why the concepts require different assessment procedures in different types of studies. In either case, the handbook should provide an explicit rationale for isolating elements of reporting quality from established systematic review concepts and evaluate whether aspects currently described as reporting quality might be better addressed at other points of the systematic review process. *[Tier 1]*[18]

EPA should incorporate these NASEM recommendations for study quality assessments, including consideration of sensitivity concepts and reporting quality separately from risk of bias, in its revised IRIS Handbook and all future IRIS assessments.

c. The protocol does not incorporate financial conflict of interest into risk of bias assessment

The National Academy of Sciences (NAS) 2014 *Review of EPA's Integrated Risk Information System (IRIS) Process* recommended that "Funding sources should be considered in the risk-of-bias assessment conducted for systematic reviews that are part of an IRIS assessment."[19]

EPA has not implemented the 2014 NAS recommendation. The protocol for the Cr(VI) assessment states that:

> Conflict of interest is not explicitly assessed because the evaluations of risk of bias and sensitivity are designed to encompass the primary aspects of methodological design that could engender concern, irrespective of the sponsoring entity.[20]

The NASEM review of the draft IRIS Handbook reiterated the importance of considering the funding source of study and again recommended accounting for funding bias in study evaluation. It is important

[17] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 62.

[18] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 62.

[19] National Research Council. Review of EPA's Integrated Risk Information System (IRIS) Process. Washington, DC: National Academies Press; 2014, page 79.

[20] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 39.

to note that the statement above from the IRIS program, explaining that it assesses study methodological design with no consideration of study sponsorship, does not respond to a key point from the NASEM which is that bias resulting from study funding can be present even after controlling for methodological biases:

> Because financial ties of the investigators can be strongly associated with favorable outcomes for the sponsors, even when taking into account other risks of bias, NRC (2014) also recommended that funding sources be considered in the risk-of-bias assessment for individual studies included in systematic reviews that are part of an IRIS assessment. That recommendation was based largely on evidence obtained from clinical studies because less was known at that time about the extent of funding bias in animal research. Since the 2014 report was published, evidence for funding bias in both the preclinical human and animal literature has increased, as discussed in Chapter 2 of this report. This recommendation has not been addressed.[21]
>
> **Recommendation 2.7:** The handbook should describe how to detect and assess the effect of funding bias on the confidence of study ratings from evidence evaluation or effect estimates from synthesis. *[Tier 1]*[22]

Importantly, including funding as a risk of bias domain does not mean the exclusion of industry-sponsored studies; it means identifying funding source as a domain of potential bias and evaluating its impact on the body of evidence.

d. The protocol retains the practice of excluding studies rated to be "critically deficient" in a single domain, and of allowing studies rated as "low confidence" to be disregarded. Contrary to NASEM recommendations, numerous studies are excluded from the Cr(VI) assessment based solely on study quality ratings.

The draft IRIS Handbook provides a choice of four ratings for each study quality domain: Good, Adequate, Deficient, or Critically Deficient. Ratings for the study quality domains are considered in assigning each study an overall rating of High, Medium, Low, or Uninformative. The Handbook then states in multiple places that any study found to be "critically deficient" in any domain of study quality are considered "uninformative" and would not be further considered in an IRIS assessment. The Handbook also states that studies rated as "low confidence" might also not be considered in the evidence synthesis in an IRIS assessment, depending on the confidence ratings of other studies in the body of evidence.

The NASEM review of the draft IRIS Handbook said that

[21] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, pages 53-54.

[22] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 30.

> A major concern is that studies that are judged as "critically deficient" or "deficient" in one evaluation domain are typically rated as "uninformative" or "low confidence" studies that are generally not considered further in the IRIS assessment.[23]
>
> Excluding studies at the study evaluation stage could lead to a substantial proportion of excluded studies due to a critically deficient rating in one domain. The importance of robust and transparent information, properly contextualized within the framework of the entire systematic review, suggests…that study evaluation ratings should not be used to exclude studies.[24]
>
> **Recommendation 4.1**: The handbook should not use the results of study evaluation as eligibility criteria for the systematic review. *[Tier 1]*[25]

In a separate report on EPA's methods for conducting systematic review under the Toxic Substances Control Act, the NASEM said

> While there is inevitably variation in the internal validity and risk of bias across individual studies, it is standard practice to include all studies, even the studies with a high risk of bias into the evidence synthesis.[26]
>
> Once a study is determined to be eligible, the study could be included in the synthesis and the risk-of-bias assessment and its limitations accounted for in any qualitative or quantitative synthesis…In the synthesis step, low-quality studies may be excluded as a sensitivity analysis, but it is inappropriate to leave them out of synthesis completely.[27]

The Cr(VI) protocol, however, retains the approach of excluding studies based on study quality ratings without modification:

> Studies with a determination of *critically deficient* in an evaluation domain will not be used for hazard identification or dose-response.[28]

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[23] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 54.

[24] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 55.

[25] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 61.

[26] National Academies of Sciences, Engineering, and Medicine 2021. The Use of Systematic Review in EPA's Toxic Substances Control Act Risk Evaluations. Washington, DC: The National Academies Press, 2021, page 35.

[27] National Academies of Sciences, Engineering, and Medicine 2021. The Use of Systematic Review in EPA's Toxic Substances Control Act Risk Evaluations. Washington, DC: The National Academies Press, 2021, page 39.

[28] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 42.

> *Uninformative* studies will not be considered further in the synthesis and integration of evidence for hazard identification or dose-response but may be used to highlight possible research gaps.[29]
>
> Studies evaluated as being *uninformative* are not considered further and, therefore, do not undergo data extraction.[30]

Additional language that is new to the Cr(VI) protocol seems intended to acknowledge the concern that a rating of critically deficient in any domain precludes use of a study, and should therefore be applied in only unusual circumstances (language that was not included in the 2019 draft protocol in **bold)**:

> *Critically deficient* reflects a judgment that the study conduct relating to the evaluation domain question introduced a serious flaw that is interpreted to be the primary driver of any observed effect(s) or makes the study uninterpretable. Studies with a determination of *critically deficient* in an evaluation domain will not be used for hazard identification or dose-response but may be used to highlight possible research gaps. **Given this potential for exclusion, this classification is used infrequently and with extreme care; methodological limitations warranting this classification are defined a priori on an exposure- and outcome-specific basis and are inherently severe enough to warrant exclusion based on a single critical deficiency.**[31]

The actual application of "critically deficient" domain ratings and "uninformative" overall study ratings in the Cr(VI) assessment, however, is quite extensive and does not display consideration of whether a flaw is "the primary driver of any observed effect(s)," as studies are routinely excluded without examination of study findings to judge how they may have been affected by methodological flaws.

In addition, the presentation of the Data Extraction step in the Cr(VI) protocol indicates that studies rated as "low-confidence" may be disregarded in conducting the assessment:

> Not all studies that meet the PECO criteria go through data extraction. Studies evaluated as being *uninformative* are not considered further and, therefore, do not undergo data extraction. In addition, outcomes that are determined to be less relevant during PECO refinement may not go through data extraction or may have only minimal data extraction. **The same may be true for *low*-confidence studies if sufficient *medium*- and *high*-confidence studies are available**…**decisions about data extraction for *low*-confidence studies are typically made during implementation of the protocol based on consideration of the quality and extent of the available evidence.**[32] (emphasis added)

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[29] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 43.

[30] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 82.

[31] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 42.

[32] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 82.

> Evidence synthesis will be based primarily on studies of *high* and *medium* confidence. *Low*-confidence studies may be used, if few or no studies with higher confidence are available, to help evaluate consistency, or if the study designs of the *low*-confidence studies address notable uncertainties in the set of *high*- or *medium*-confidence studies on a given health effect. If *low*-confidence studies are used, then a careful examination of risk of bias and sensitivity with potential impacts on the evidence synthesis conclusions will be included in the narrative.[33]

The practice of excluding "low-confidence" studies is also contrary to the NASEM recommendation that the IRIS program "should not use the results of study evaluation as eligibility criteria for the systematic review."[34] Instead, all studies should be considered in evidence synthesis and the "careful examination of risk of bias and sensitivity with potential impacts on the evidence synthesis conclusions will be included in the narrative," should be the approach applied to all studies.

The draft Cr(VI) assessment repeatedly (across health outcomes) and automatically excludes studies with a critically deficient rating from further consideration. In each category of health effects for which PECO-relevant epidemiological studies were identified, at least one of these studies was not considered due to an "uninformative" rating (Table 1). For some categories a substantial proportion of studies were excluded based on the study quality rating; for example, 5 out of 10 studies of respiratory effects were excluded due to an "uninformative" classification. In addition, large numbers of animal studies were similarly excluded from consideration for some effect categories: 6 out of 21 animal studies of hematologic effects, and 7 out of 27 animal studies of male reproductive effects were classified as "uninformative."

Classification of studies as "uninformative" based solely on examination of methods precludes any consideration of the study results. Results of such studies may in fact provide useful information to the assessment; for example, findings of adverse effects even in studies with "critically deficient" exposure misclassification or sensitivity (which may be biased towards the null) may be considered to increase the strength of evidence for the outcome in question. The IRIS approach does not allow for such consideration.

The Cr(VI) draft assessment does appear to include all "low confidence" animal and human studies in its evidence syntheses. While the low confidence studies were not disregarded, they were frequently discounted without adequate justification (see comments below).

[33] U.S. EPA. Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment (Preliminary Assessment Materials). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191b, External Review Draft, 2022, page 91.

[34] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 61.

Table 1. Number of epidemiological studies of hexavalent chromium classified as "uninformative," by health effect

Health effect category	Total number of PECO-relevant epidemiological studies	Number of PECO-relevant epidemiological studies classified as "uninformative"
Respiratory Tract Effects Other Than Cancer	10	5
Gastrointestinal Tract Effects Other Than Cancer	0	N/A
Hepatic effects	4	1
Hematologic effects	5	1
Immune effects	13	4
Male Reproductive Effects	5	2*
Female Reproductive Effects	3	2
*For the category of Male Reproductive Effects, two studies were rated "uninformative" for some endpoints and "low confidence" for other endpoints.		

2. Evidence synthesis and integration for non-cancer outcomes should be improved.

a. The distinction between evidence synthesis and evidence integration should be clarified

The Cr(VI) protocol continues the lack of clarity found in the draft IRIS Handbook regarding the distinction between evidence synthesis (which refers to conclusions from a particular stream of evidence) and evidence integration (which refers to conclusions considered from the combination of evidence streams). The NASEM review of the Handbook commented that:

> The terms "synthesis," "integration," and "strength of the evidence" appear to be used almost interchangeably, when in fact these should be distinct steps in the systematic review process.[35]
>
> Another example of a need for improved organization of the handbook is the overlap between Chapter 9, "Analysis and Synthesis of Human and Experimental Animal Data," and Chapter 11, "Evidence Integration." Both chapters list considerations for evidence synthesis within a group of outcomes of human or animal evidence (e.g., Table 9-1 (p. 9-3) and Table 11-2 (p. 11-10)), but the criteria are not the same in each chapter. Only Chapter 11 describes how the

[35] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 71.

ratings for each of these considerations produces a strength of evidence judgment for a body of human or animal evidence, which is then advanced to the evidence integration step.[36]

Finding: The handbook is confusing as to the transition from the synthesis step (within a data stream) to integration (across data streams), in particular because many considerations for synthesis are repeated (with slight variation) in Chapters 9 and 11.[37]

The Cr(VI) protocol maintains the confusing presentation of the synthesis and integration steps from the draft IRIS Handbook, and the assessment would be improved by clearer separation of the steps of drawing evidence synthesis conclusions and evidence integration conclusions, as recommended by the NASEM.

b. Evidence synthesis classifications appear to understate the strength of the evidence for some health outcomes

The synthesis of evidence regarding non-cancer effects of Cr(VI) is often lacking in clarity, and justifications for the hazard classifications are often incomplete or insufficient.

For example, in the assessment of hepatic effects, the draft concludes that the animal evidence for histopathology is "moderate." Ample evidence of histopathology is presented:

> Elevations of ALT and AST were seen across the oral evidence base, with biologically significant elevations in ALT (>100%) seen in multiple studies. ALT in particular is considered a sensitive and specific indicator of liver injury…Dose-dependent increases in chronic inflammation…in female F344 rats exposed for three months to two years…vacuolation and fatty changes were also observed in several studies…several lower confidence subchronic studies in rats noted increased evidence of apoptosis or necrosis…supported by mechanistic evidence that 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. 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).[38]

The only noted limitation of the evidence base is the absence of increased necrosis in studies rated as high-confidence. Even so, increased necrosis is observed in other studies and "numerous mechanistic

[36] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 20.

[37] National Academies of Sciences, Engineering, and Medicine. Review of U.S. EPA's ORD Staff Handbook for Developing IRIS Assessments: 2020 Version. Washington, DC: The National Academies Press, 2022, page 68.

[38] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, pages 3-178 to 3-179.

studies have indicated an increase in necrosis and markers of apoptosis."[39] Overall, the narrative synthesis of the evidence indicates consistent findings of adverse and toxicologically significant effects that support a classification of "robust" evidence.

The synthesis of animal evidence for immune effects also raises questions regarding the choice of classification. In this case, the evidence was rated as "slight" even though it includes:

> evidence of effects on ex vivo WBC function…antibody responses to T cell-dependent antigen… 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.[40]

> Cr(VI) induced changes in the most meaningful immunological endpoint (i.e., antibody response, host resistance and ex vivo WBC function) and endpoints that provide supporting evidence (i.e., immune organ weight, immunoglobulin levels, and WBC counts).[41]

The summary narrative does not further consider the reasons that some studies were rated as "low confidence" and whether the specific shortcomings leading to that rating actually decrease confidence in their findings, nor does it evaluate possible explanations for inconsistency. Rating of studies as "low confidence" should not automatically result in a classification of "slight" evidence when there are multiple studies supporting a hazard conclusion across different laboratories, species, endpoints and routes of exposure. It appears that the animal evidence supports a classification of "moderate" evidence.

Similar shortcomings in the selection of an evidence classification are seen with the designation of the human evidence for male reproductive effects as "slight." The narrative describes consistent evidence of adverse effects:

> Occupational (inhalation) Cr(VI) exposure is inversely associated with sperm concentration, normal sperm morphology, sperm motility, and serum testosterone.
> These findings are consistent and coherent across multiple studies and endpoints, but interpretation is limited because most studies evaluating sperm were considered low confidence.[42]

[39] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, page 3-180.

[40] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, page 3-225.

[41] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, pages 3-228 to 3-229.

[42] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, page 3-256.

The only rationale offered for designating the evidence as "slight" is that studies were rated as "low confidence," but again no examination of the particular flaws noted in the study quality evaluation is applied in selecting the evidence classification. The summary narrative supports a classification of "moderate" human evidence.

3. Selection of studies for dose-response analysis and derivation of candidate RfDs is clear and well-justified. However, insufficient justification is provided for selection of GI tract effects rather than liver effects for the overall RfD.

Dose-response analysis was conducted for GI tract, liver, developmental, and hematological endpoints and candidate RfD values are presented in Table 4-4. EPA selected the value of 9×10^{-4} for GI tract effects as the overall RfD. This value, however was not the lowest candidate RfD shown in the table:

- The candidate RfD for chronic liver inflammation from the 2-year NTP study in female rats is 6.69×10^{-4} mg/kg-day (LOAEL = 0.0669 mg/kg-day, composite UF = 100).
- The candidate RfD for GI tract hyperplasia from the 2-year NTP study in female mice is somewhat greater at 9.11×10^{-4} mg/kg-day (LOAEL = 0.0911 mg/kg-day, composite UF = 100).

The draft assessment acknowledges that the RfD for liver effects is lower, but does not present an adequate rationale for selecting the GI tract effect for the overall RfD:

> While the osRfD for liver was slightly lower, the osRfD for GI effects is still lower than most other candidate values considered for the liver osRfD (see Figure 4-3). With the exception of chronic liver inflammation in female rats, candidate values for the osRfD for liver effects that were based on chronic exposure data (12 months or 2 years; see Figure 4-3) were above 9×10^{-4} mg/kg-d. Candidate liver values derived from subchronic data that were lower than 9×10^{-4} mg/kg-d had cumulative uncertainty factors of 300, whereas other candidate values had uncertainty factors of 100 or less. Because the GI tract is exposed to higher concentrations of un-reduced Cr(VI) than the liver, it is likely to be more susceptible to the effects of ingested Cr(VI). Thus, the osRfD for GI effects was selected as the overall RfD.[43]

This paragraph seems to argue that the lowest candidate RfD for liver effects was not selected as the overall RfD because other candidate values for liver effects were higher. It also speculates that the GI tract is "likely to be more susceptible to the effects of ingested Cr(VI)." Neither point explains why the value for chronic liver inflammation in female rats (which, by the RfD methodology appears to indicate greater susceptibility) was not selected as the overall RfD.

[43] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, page 4-20.

4. EPA's conclusions regarding carcinogenicity of Cr(VI) by the oral route of exposure are well-supported.

The draft assessment presents a careful review of the evidence regarding carcinogenicity of Cr(VI) from oral exposures, including extensive discussion of genotoxicity evidence and a mode-of-action analysis. The assessment concludes that:

> **Cr(VI) is likely to be carcinogenic to humans** via the oral route of exposure. Robust evidence shows tumors of the GI tract in mice (small intestine) and rats (oral cavity) in both sexes; the oral cavity tumors were rare indicating increased biological significance. Evidence from humans is slight but is consistent in reporting some risk of cancers of the GI tract in humans exposed via drinking water…A mutagenic mode of action for Cr(VI) carcinogenicity is considered sufficiently supported in (laboratory) animals and relevant to humans.[44]

This conclusion is consistent with the relevant evidence from the NTP bioassays and mechanistic studies. EPA carefully assembled and evaluated all of the relevant evidence to make a strongly-supported inference that there is a mutagenic mode of action for cancer by both inhalation and oral exposure.

[44] U.S. EPA. IRIS Toxicological Review of Hexavalent Chromium (External Review Draft, 2022). U.S. Environmental Protection Agency, Washington, DC, EPA/635/R-22/191, 2022, pages 3-157 to 3-159.