Draft Charge for the
Toxicological Review of Hexavalent Chromium
October 2022

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of the draft *IRIS Toxicological Review of Hexavalent Chromium* developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's Center for Public Health and Environmental Assessment within the Office of Research and Development. IRIS assessments contain information about chemicals that encompasses hazard identification and 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, IRIS assessments support the Agency's regulatory activities and decisions to protect public health.

This assessment updates a previous 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 draft Toxicological Review of Cr(VI) is based on a comprehensive 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 are provided as *Supplemental Information—Appendix A: Systematic Review Protocol for the Hexavalent Chromium IRIS Assessment* and *Supplemental Information—Appendices B, C, D, and E* to the draft Toxicological Review.

Charge Questions on the Draft Toxicological Review of Cr(VI)

When responding to the charge questions below, categorize any recommendations for EPA as part of this peer review into one of three categories (Tier 1, 2, or 3). The categorized comments are useful for prioritizing the relative importance of comments, as follows:

- Tier 1: *Necessary Revisions* – Use this category for any revisions you believe are necessary to adequately support and substantiate the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the Cr(VI) Toxicological Review
- Tier 2: *Suggest* – Use this category for any revisions you encourage EPA to implement to strengthen the analyses or scientific basis for the assessment conclusions, or to improve the clarity of the presentation in the Cr(VI) Toxicological Review
- Tier 3: *Future Considerations* – Use this category for any advice you have for scientific exploration that might inform future work. While these recommendations are generally outside the immediate scope or needs of the Cr(VI) Toxicological Review, they could inform future reviews or research efforts.

1. 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].
2. 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. 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.
    b. 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.

Noncancer Hazard Identification and Toxicity Value Derivation

3. 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. 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.
    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

[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.

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

primarily based on robust studies in rodents that found Cr(VI) causes non-neoplastic effects in the GI tract.

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.

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.

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.

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.

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 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.

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.

   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.

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.

   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.

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).

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.

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.

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.

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.

4. 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?

5. 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.

    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?

    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?

    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?

Carcinogenicity Hazard Identification and Toxicity Value Derivation

6. 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. 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.

    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 alow-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).

    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).

    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.

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).