ATSDR has consulted the EPA IRIS Toxicological Review of Hexavalent Chromium, Supplemental Materials, and Draft Peer Review Charge. Below are ATSDR responses to the Peer Review Charge Statements/Questions. Additional comments are annotated on the Toxicological Review and Supplemental Materials. Some BMD analyses are also being submitted for EPA consideration.

1. For the most part, the literature search strategy and screening criteria are appropriate, though some mention of telomeres would be suggested in the cancer section of the Toxicological Review; see #6 response, below.

No additional peer reviewed chromium VI studies have been identified.

2.a. Please refer to the annotated comments of the Toxicological Review for comments on confidence conclusions.
2.b. Except for annotated comments of the Toxicological Review on scientific support for the confidence conclusions, EPA was very thorough in addressing study results. The one conclusive judgment on hematological effects seems more subjective given the database weaknesses.

3.a. Gastrointestinal (noncancer)

i. ATSDR agrees that Cr (VI) likely causes gastrointestinal (GI) tract toxicity.

ii. Agree with developing cRfD from the NTP (2008) study based on diffuse epithelial hyperplasia in the female mouse small intestine. The study, species, endpoint, and final cRfD value is in alignment with ATSDR's Chromium Toxicological Profile (2012) and chronic inhalation minimal risk level (MRL) for chromium (VI). ATSDR did use an older version of Benchmark Dose (BMD) software to arrive at a point of departure that was then divided by a total uncertainty factor (UF) of 100; The ATSDR total UF multiples a 10 UF for human variability by a 10 UF for animal to human extrapolation. EPA converted the LOAEL to an internal dose, time weighted average, and allometrically adjusted. This was then converted to a human equivalent dose and a composite UF of 100 (3 for intraspecies;10 for LOAEL to NOAEL; and 3 for animal to human) was applied. After calculations, the EPA value and ATSDR's value (based on BMD) were the same.

iii(1). Yes, the NTP (2008) study based on diffuse epithelial hyperplasia of the duodenum in the female mouse small intestine is appropriate for the organ-specific (OS) RfD for GI toxicity.

iii(2). No, the OS RfD should not be based on the male mice diffuse epithelial hyperplasia of the duodenum as the resultant final RfD value is not as protective as the female data. Another reason is there appears to be sex differences with toxicity.

iii(3). No, a combination of PODs from both sexes is not scientifically justifiable.

iii(4). The endpoint and datasets for the endpoint are appropriately used. However, please review the annotated comments on the Hexavalent Chromium Toxicological Review and Supplemental Materials for modeling comments. Also included are the output files from BMDS v3.2 modeling for the diffuse hyperplasia of duodenum endpoints using the data specified in the Supplemental Material, page D-2. It does not appear that EPA used unrestricted models in assessing the datasets. Also, while there is much discussion on why BMD was not considered useful for the female mice, there is no discussion on why the

results obtained from the male mice and why those were not considered viable. At a minimum, ATSDR suggests a re-run of the data in BMDS v3.2 to ascertain whether the program is giving consistent results for frequentist restricted and unrestricted models (see attached Excel spreadsheets for model runs).

3.b. Respiratory (noncancer outside the nasal cavity)

i. Agree that evidence indicates inhaled chromium VI [CR (VI)] likely causes lung (lower respiratory tract) effects. EPA does a good job in summarizing the database for these findings and provides mechanistic support. ATSDR has likewise indicated the lung as a target for Cr (VI) toxicity (ATSDR 2012).

ii. Glasser et al. (1990) is the study selected for an ATSDR intermediate inhalation MRL and EPA OS RfC. ATSDR identified a change in lactate dehydrogenase as the critical effect. ATSDR arrived at an MRL of 3 x 10^{-4} mg/m^3 by utilizing BMD and converting to a human equivalent concentration. ATSDR used a total uncertainty factor of 30 that accounted for animal to human extrapolation (3 UF) and human variability (10 UF). EPA indicated that BMD was not amenable for many lung endpoints and thus used a LOAEL (133 µg/m^3) for cellular response and histopathology was used in the calculation of the OS RfC. EPA utilized a rather large composite UF of 1,000 to arrive at their final value for an OS cRfC of 1 x 10^{-4} mg/m^3. EPA justified a database deficiency UF of 3 because portal of entry effects are the most pharmacokinetically sensitive. However, ATSDR does not feel the database for the effect is lacking as lung effects are observed in both huma (lung function decline) and animal (cellular response and histopathology) data. Suggest that EPA remove the database UF when calculating the OS RfC. Similar to ATSDR, the interspecies UF was reduced from 10 to 3 because of converting to a human equivalent concentration. The LOAEL UF was reduced to a 3 because cellular and pathological response are sensitive indicators of lung injury and are resolvable.

3.c. Respiratory (noncancer nasal cavity)

i. Agree that evidence demonstrates that inhalation of Cr (VI) may cause nasal lesions. ATSDR developed an intermediate-duration inhalation MRL of 5 x 10^{-6} mg Cr(VI)/m^3 for this endpoint based on Lindberg and Hedenstierna 1983. The ATSDR intermediate-duration inhalation MRL for chromium (VI) is based on a lowest observable effect level of 2 x 10^{-3} mg/m^3 that was duration adjusted to 5 x 10^{-4} mg/m^3 and divided by a total UF of 100 (10 for use of a LOAEL and 10 for human variability). EPA's OS cRfC is 1 x 10^{-5} mg/m^3 based on ulceration of the nasal septum (Gibb et al. 2000a) and a composite UF of 300 (3 for database deficiency, 10 for use of LOAEL, 3 intraspecies extrapolation [UF_H], and 3 for subchronic to chronic duration extrapolation). ATSDR does not believe a UF of 3 for database deficiency is necessary as nasal ulceration/atrophy/perforation are consistent findings in epidemiological (Lindberg and Hedenstierna 1983; Gibb et al. 2000a) and animal studies (Adachi 1987; Adachi et al. 1986; Nettesheim and Szakai 1972; see ATSDR Chromium Toxicological Profile for those references). Using a UF of 3 for intraspecies extrapolation instead of 10 is also questionable. EPA indicated this was based on sex and it being a portal of entry effect. ATSDR suggests a UF of 10 to be most protective of the female sex.

3.d. Hepatic

This is an interesting study where the authors have investigated the effects and mechanisms of Cr (VI) exposures (NTP 2008) with important implications for many individuals exposed to Cr (VI).

i. Three studies corelating Cr (VI) exposure (Saraswathy and Usharani, 2007; Lin et al., 1994 and Sazakli et al., 2014) and liver clinical chemistries are discussed here as a markers of liver damage and cholestasis.

The review is technically fine; however, suggest discussion of the below in this section:

- The bio-persistence and clearance of Cr (VI) data in-vivo (liver). This is critical because as discussed in 3.2.4.3. inhaled and ingested Cr (VI) can accumulate in the liver. Also, damaged tissue has been reported to infiltrate macrophages (Yamate et al., 2016), these macrophages were significantly elevated in female rats as observed in 3 months and 2 years study (NTP 2007 and 2008).
- Whether macrophages are involved in clearing Cr (VI) from the tissue.
- Whether there is any data available on metal content in the liver at the different time points discussed.

ii. The idea of calculating cRfD, based on Cr (VI)-induced chronic inflammation of female F344 rats in 2 years drinking water bioassay is well defined (NTP 2008).

3.e. Hematological

ATSDR derived a 5 x 10^{-3} mg/kg/day intermediate-duration oral MRL from the anemia endpoint that occurred in rats (NTP 2008). The BMDL-2 standard deviations for multiple hematological endpoints were averaged to 0.52 mg/kg/day and divided by a total UF of 100 (10 for animal to human extrapolation and 10 for human variability). EPA has not developed an OS cRfD for this health effect because the evidence judgment was not 'evidence indicates' but instead 'evidence suggests' (a lower judgment) and only the higher judgments were considered for developing OS cRfDs. EPA states that the finding returns to normal or near normal levels by 12 months. ATSDR chose to derive an MRL because anemia was observed over multiple time points (22 days to 6 months). The ATSDR MRL was developed because the health effect would make an organism susceptible to other toxic insults. It is only for the intermediate duration (15 to 364 days). By limiting the duration of the MRL, ATSDR accounts for the finding of an un-sustained effect beyond 365 days.

3.f. Immune

ATSDR agrees with the EPA evidence judgment of 'evidence suggests' that Cr (VI) may influence the immune system.

3.g. Male reproductive

ATSDR agrees with the EPA evidence judgment of 'evidence suggests' that Cr (VI) may cause male reproductive toxicity.

3.h. Female reproductive

ATSDR agrees with the EPA evidence judgment of 'evidence inadequate' for female reproductive effects caused by Cr (VI) exposure.

3. Developmental

i. In this section both direct and indirect routes of Cr (VI)- exposure to developing organism are well described. Overall, this is an interesting and well conducted section. Conclusions drawn by authors are well supported as evidenced by the experimental data discussed here.

Significantly increased spontaneous abortion was reported after stainless-steel welding but not with mild steel welding exposure (Hjollund et al., 2000). One of the reasons to explain this could be stainless-steel welding are significantly more water soluble and may cause more persistent and greater inflammatory response as compared to mild steel welding exposures. Please refer to Shoeb, M., Kodali, V.K., Farris, B.Y., et al., 2017. Oxidative Stress, DNA methylation, and telomere length changes in peripheral blood mononuclear cells after pulmonary exposure to metal-rich welding nanoparticles. NanoImpact 5, 61–69.

- Page 3-297, line 18, "decreased maternal body weight gain but no effect on placenta weights." Please add a reference to support the sentence.

ii. Decreased F1 offspring growth and stomach ulcers in rats after exposure to high maternal doses of 24.4 and 20 mg/kg-d was observed. These high concentrations (in low confidence studies) were reported to cause other toxic effects as well. The selection of utilizing the continuous breeding study (NTP, 1997) to derive reference oral dose is justified and well discussed.

4. Use of BMD

See the Toxicological Review of Hexavalent Chromium [Cr(VI)] Supplemental Information document for ATSDR comments.

5. Uncertainty factors (UF)

a. UFs adequate

Using UFs to calculate the OS cRfDs and RfCs is standard practice. See the endpoint specific responses for number 3 and whether UFs have been adequately addressed.

b. Intraspecies variability

See the endpoint specific responses for number 3 and whether the UF value is challenged.

c. Database deficiency UF of 3 inhalation respiratory effects (upper and lower)

See the endpoint specific responses for number 3 and whether the UF value is challenged.

5.d. Subchronic to chronic UF of 3 for nasal effects
See the endpoint specific responses for number 3 and whether the UF value is challenged. The charge document's description of why this was lowered is better than what is suggested in a Toxicological Review Section (~ 4).

6. Cancer

In the current review, authors have nicely demonstrated the carcinogenic and mutagenic effect of Cr (VI) exposure. The majority of experimental data and genotoxic events have been appropriately discussed in this review however, telomere alteration is not discussed. Below are a couple of references that may be used and discuss telomeres. There are several sources of Cr (VI) exposure in occupational sectors. Stainless steel welding fume is a complex mixture of different metals, such as chromium (Cr), manganese (Mn), nickel (Ni), and iron (Fe) and alteration in telomere length homeostasis after welding fumes exposure (intratracheal instillation and inhalation) have been reported in vivo. Although welding fumes exposure will not be indicative of which potential/specific metals present in the fumes is responsible for dysfunctional telomeres.

Shoeb, M., Kodali, V.K., Farris, B.Y., et al., 2017. Oxidative Stress, DNA methylation, and telomere length changes in peripheral blood mononuclear cells after pulmonary exposure to metal-rich welding nanoparticles. NanoImpact 5, 61–69.

Shoeb, M., Mustafa, G.M., Kodali, V. K., et al. (2020). A possible relationship between telomere length and markers of neurodegeneration in rat brain after welding fume inhalation exposure. Environmental Research 180, 108900.

a. EPA concluded mutagenic and carcinogenic potential of Cr (VI) based on available human and animal data which is in line with the chromium tox profile (ATSDR 2012). Human oral exposure to Cr (VI) was limited to three low confidence (due to limitations in the exposure assessment) studies utilizing ecological analysis of cancer mortality after Cr (VI) contamination in drinking water. However, there is plenty of evidence of human exposure to Cr (VI) via inhalation route which have been nicely discussed suggesting an increased risk of GI tract cancer in occupationally exposed workers. Oral exposure to Cr (VI)-induced GI cancer in rats and mice was also discussed in tox profile of chromium (ATSDR 2012).

b. In the gastric environment Cr (VI) is reduced to Cr (III) thereby limiting the bioavailability of Cr (VI). As suggested, potential of toxicological effect of Cr (VI) will be different at portal and systemic tissue depending upon the routes of exposures. Therefore, assumption of low-dose linearity for both routes of exposure (oral and inhalation) sounds relevant.

c. Agree that the oral route of Cr (VI) exposure is likely to be carcinogenic to humans based on NTP 2008. The efficacy of Cr (VI) to cause carcinogenic lesions depends on its route (oral vs inhalation) of exposure. Likewise, ATSDR (2012) indicates the GI and respiratory tracts are suggested to be most sensitive to such exposures.

d. Agree with using NTP 2008 male and female mice and incidence of small intestine carcinomas and adenomas to calculate the total lifetime OSF for CR(VI) of 0.5 mg/kg/day since the nonlinear reference oral dose estimate would be 4.5x higher.

e. Agree with the high and medium confidence studies (Gibb 2020, 2015, 2000b and Proctor et al., 2016) considered for IUR development for Cr (VI).