Memorandum

Date: December 7, 2020

To: Mark Benton, Deputy Secretary for Health Services, NC Department of Health and Human Services

Shelia Holman, Assistant Secretary for the Environment, NC Department of Environmental Quality

From: Tom Augspurger, PhD
Chair, Secretaries' Science Advisory Board

Subject: Secretaries' Science Advisory Board response to inquiry on hexavalent chromium

Background

Two duties of the Secretaries' Science Advisory Board (SSAB) are to act as consultants to the North Carolina Department of Environmental Quality (DEQ) on factors for establishing acceptable levels of contaminants and to provide input to the North Carolina Department of Health and Human Services (DHHS) as they establish health goals. In June 2018, DEQ and DHHS requested the SSAB's review and recommendations on hexavalent chromium [Cr(VI)] science to use for developing public health and environmental standards. In December 2018, the charge to the SSAB was refined as follows:

> *DEQ and DHHS requests the SSAB review the current hexavalent chromium toxicological science related to a linear versus a non-linear exposure response and provide recommendations to the appropriate science to be used for development of regulatory standards protective of public health and the environment for groundwater and surface water.*

This memorandum conveys the SSAB's response to that specific charge.

A decision to select a linear or a non-linear dose-response model for oral exposures to Cr(VI) is informed by consideration of the toxicological and epidemiological evidence, particularly as it informs mode(s) of action. A mutagenic mode of action in carcinogenesis would typically lead to assumption of a linear no-threshold approach to dose-response assessment

(resulting in calculation of an oral slope factor, OSF) whereas a non-mutagenic (e.g., effects due to cytotoxicity) mode of action would typically lead to assumption of a non-linear approach based on identification of a point of departure and application of uncertainty factors (resulting in an estimate of a reference dose, RfD). At low doses a mutagenic mode of action may be operative whereas at higher doses cytotoxicity or other mechanisms may be operative. Therefore both mutagenic and cytotoxic modes of action may result from chemical exposure with mutagenicity occurring at all levels of exposure and as the putative mode of action in the low-dose region. There are different lines of evidence emerging for, and different published perspectives on, Cr(VI) mode of action, and results from RfD versus OSF approaches to deriving estimates of health protective drinking water concentrations vary by orders of magnitude.

Approach and Analysis

The SSAB received scientific data and information from Federal, State, and international government agencies, from a consulting company to industry stakeholders, and by members of the public. The North Carolina DEQ and DHHS, Texas Commission on Environmental Quality, New Jersey Department of Environmental Protection, California Environmental Protection Agency, ToxStrategies, and Health Canada made presentations to the SSAB. The materials presented and a summary of the discussions during the presentations are found on the SSAB website (https://deq.nc.gov/about/boards-and-commissions/secretaries-science-advisory-board). The reader is directed to that publicly available website for specific information as well as audio files of the presentations and discussions. The information provided to the SSAB was useful but note that a critical review of the presentations has not occurred, nor has the SSAB conducted a detailed quality evaluation of all the scientific studies summarized below.

The SSAB approved a draft hexavalent chromium recommendation document be sent for public comment in February 2020. The draft recommendations were subsequently posted for public comment through June 1, 2020. Four sets of comments were received and all were shared in their entirety with SSAB members on June 15th. The SSAB discussed the comments during the August 2020 board meeting, and nine comments were flagged for follow up. These

comments questioned interpretation and/or consistency with references cited in the SSAB's draft recommendations. Research into these comments was completed in September 2020 and shared with SSAB members in advance of their October meeting when SSAB members reviewed comments, consistency with original references, and suggested how to address comments in the final recommendations. The review comments and notes of the SSAB's deliberate evaluation of them are attached.

The SSAB's review focused on research, reviews, and syntheses conducted over the last fifteen years, a period of active investigation on the mode or modes of action of Cr(VI) toxicity following National Toxicology Program (NTP 2007 and 2008) drinking water studies in mice and rats which reported tumors evidencing carcinogenic activity and other effects. The SSAB reviewed independently and discussed current literature and recent syntheses related to hazard assessment of Cr(VI) in drinking water. We note the value of recent syntheses (e.g., McCarroll et al. 2010; Stern 2010; USEPA 2010; ATSDR 2012; Zhitkovich 2011; Haney 2015a-c; Sun et al. 2015; Health Canada 2016; Thompson et al. 2013, 2014, 2017a, 2018; Suh et al. 2019) which examine and evaluate the weight of evidence for linear and non-linear modeling approaches to existing data as the most relevant to the charge from DEQ and DHHS. There are also highly relevant mode of action studies (e.g., O'Brien et al. 2013; Thompson et al. 2015a-c, 2017b; Aoki et al. 2019), many but not all of which are referenced in the hazard assessment syntheses. With over 1,000 potentially relevant papers on Cr(VI) mode of action, each new synthesis has the opportunity to build on recent data. We note an on-going systematic review of the mutagenic potential of orally administered Cr(VI) (USEPA 2019) as an opportunity to have refinement of the following analysis and recommendations when the USEPA analysis is completed.

We derived recommendations following the USEPA's Guidelines for Carcinogen Risk Assessment (USEPA 2005) and Guidelines for Mutagenicity Risk Assessment (USEPA 1986). The 2005 USEPA guidelines state:

> "When the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach. Nonlinear approaches generally should not be used in cases where the mode of action has not

been ascertained. Where alternative approaches with significant biological support are available for the same tumor response and no scientific consensus favors a single approach, an assessment may present results based on more than one approach.

A *nonlinear* approach should be selected when there are sufficient data to ascertain mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. 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.

Both *linear and nonlinear* approaches may be used when there are multiple modes of action. If there are multiple tumor sites, one with a linear and another with a nonlinear mode of action, then the corresponding approach is used at each site. If there are multiple modes of action at a single tumor site, one linear and another nonlinear, then both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges. For example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity does not occur. Modeling to a low response level can be useful for estimating the response at doses where the high-dose mode of action would be less important. "

Because there is evidence in the material we reviewed for both linear and non-linear quantitative approaches in modeling the oral exposures to Cr(VI), we evaluated current support for each below and conclude with a discussion on the weight of the evidence for each.

Cancer and other endpoints in key primary references

Evidence regarding Cr(VI) carcinogenesis comes from both human epidemiological and animal studies. For example, Cr(VI) is a recognized human carcinogen following ~~with mutagenic action in~~ inhalation exposures with mechanisms that include the induction of DNA damage (IARC 2012). The NTP has classified Cr(VI) as a known human carcinogen based on sufficient evidence of carcinogenicity from studies in humans (NTP Report on Carcinogens, Fourteenth Edition see: https://ntp.niehs.nih.gov/ntp/roc/content/profiles/chromiumhexavalentcompounds.pdf). This determination is largely based on occupational cohorts exposed to Cr(VI) via inhalation.

A two-year NTP (2008) bioassay exposed male and female rats and mice to dichromate dihydrate in drinking water. Rats were exposed to drinking water containing 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate (equivalent to 0, 5, 20, 60, or 180 mg/L hexavalent chromium) for 2 years (equivalent to average daily doses of approximately 0.6, 2.2, 6, or 17 mg sodium dichromate dihydrate/kg body weight for males and 0.7, 2.7, 7, or 20 mg/kg for females). Male mice were exposed to drinking water containing 0, 14.3, 28.6, 85.7, or 257.4 mg/L sodium dichromate dihydrate (equivalent to 0, 5, 10, 30, or 90 mg/L hexavalent chromium) for 2 years (equivalent to average daily doses of approximately 1.1, 2.6, 7, or 17 mg sodium dichromate dihydrate/kg body weight). Female mice were exposed to drinking water containing 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate (equivalent to 0, 5, 20, 60, or 180 mg/L hexavalent chromium) for 2 years (equivalent to average daily doses of approximately 1.1, 3.9, 9, or 25 mg/kg hexavalent chromium).

Exposure of rodents to Cr(VI) was associated with decreased body weight and water consumption that was secondary to palatability issues. Mean body weights of 516 mg/L sodium dichromate dihydrate (180 mg/L hexavalent chromium) males and female rats were less than those of the controls throughout the study. Water consumption by 172 and 516 mg/L sodium dichromate dihydrate rats was less than that by the controls throughout the study. Terminal mean body weight of 172 mg/L sodium dichromate dihydrate (60 mg/L hexavalent chromium) female mice was 8% less than that of the controls, and the mean body weight of 516 mg/L female mice was 15% less than that of the controls. Water consumption by 85.7 and 257.4 mg/L sodium dichromate dihydrate males and 172 and 516 mg/L sodium dichromate dihydrate female mice was less than that by the controls throughout the study.

NTP reported tumors rodents exposed via drinking water to Cr(VI). Exposure to sodium dichromate dihydrate resulted in the development of squamous cell carcinoma in the oral mucosa of male and female rats in the highest exposure group (516 mg/L). An increased incidence of oral squamous cell carcinoma was also seen in female rats in the 172 mg/L exposure group. The incidences of squamous cell papilloma or squamous cell carcinoma (combined) of the oral mucosa or tongue of 516 mg/L male and female rats were significantly greater than those in the controls.

Neoplasms of the small intestine (duodenum, jejunum, or ileum) were seen in exposed male and female mice. The incidences of adenoma of the duodenum in 257.4 mg/L males and 172 and 516 mg/L female mice were significantly greater than those in the controls. The incidence of carcinoma of the duodenum was statistically significantly increased in 516 mg/L female mice. The incidence of adenoma of the jejunum in 516 mg/L female mice was significantly increased compared to that in the controls. When the incidences of adenoma and carcinoma tumors were combined for all sites of the small intestine, the incidences were statistically significantly increased in 85.7 and 257.4 mg/L males and 172 and 516 mg/L females compared to those in the controls. The incidences in 57.3 mg/L females exceeded the historical control ranges for drinking water studies and for all routes of administration. The incidences of diffuse epithelial hyperplasia were significantly increased in the duodenum of all exposed groups of male and female mice. The incidences of histiocytic cellular infiltration were significantly increased in the duodenum of 85.7 and 257.4 mg/L males and in 172 and 516 mg/L females. In the jejunum, the incidences of diffuse epithelial hyperplasia and histiocytic cellular infiltration were significantly increased in 516 mg/L females. The incidences of histiocytic cellular infiltration of the liver in all exposed groups of females, of the mesenteric lymph node in all exposed groups of males and females, and of the pancreatic lymph node of 85.7 and 257.4 mg/L males and 172 and 516 mg/L females were significantly increased.

Exposure concentration-related non-neoplastic liver lesions including but not limited to histiocytic cellular infiltration and chronic inflammation were observed in male and female rats exposed to ≥ 57.3 mg/L. Increased incidences of histiocytic cellular infiltration also occurred in the small intestine (duodenum), mesenteric lymph node, and pancreatic lymph node of males and/or females exposed to ≥ 57.3 mg/L. Microcytosis occurred in exposed mice; the mice were less affected than the rats.

The NTP (2008) concluded that there was clear evidence of carcinogenic activity of sodium dichromate dihydrate exposure via drinking water in male and female F344/N rats based on increased incidences of squamous cell neoplasms of the oral cavity. There was clear evidence

of carcinogenic activity of Cr(VI) associated with the sodium dichromate dihydrate exposure in male and female B6C3F1 mice based on increased incidences of neoplasms of the small intestine (duodenum, jejunum, or ileum). Exposure to sodium dichromate dihydrate also resulted in histiocytic cellular infiltration in the liver, small intestine, and pancreatic and mesenteric lymph nodes of rats and mice and diffuse epithelial hyperplasia in the small intestine of male and female mice.

Dose-response modeling

This section focuses on issues pertinent to disposition of chromium in the body and dose-response for the oral route of exposure. Chromium, like many other metals, undergoes valence state shifts rather than enzymatically catalyzed biotransformation. Trivalent chromium [Cr(III)] is an essential element associated with carbohydrate metabolism, whereas Cr(VI) is classified as a known human carcinogen in the lung. Gastric juices reduce Cr(VI) to Cr(III) via a 2^{nd}-order reaction *in vitro*. Total reducing capacity in all mammalian species is generally between 10–30 mg/L gastric contents. Components of gastric juice reducing Cr(VI) include ascorbate, glutathione, NADH, and sulfhydryls. Reduction rate decreases as pH increases (De Flora et al. 1997; Proctor et al. 2012; Kirman et al. 2013). This is an important consideration due to differences in stomach structure and pH between rodents and humans. Transport of Cr(VI) occurs rapidly by unspecified phosphate and sulfate active transporters (Alexander and Aaseth 1995) whereas transport of Cr(III) occurs more slowly via diffusion. Gastrointestinal absorption rates are highly variable for both Cr(VI) and Cr(III). Uptake of Cr(VI) from the gut lumen is rapid and systemic reduction to Cr(III) is also rapid. Once reduced, Cr(III) will diffuse slowly into or out of tissues, and distribute to tissues in plasma.

Both the uptake and reduction of Cr(VI) by red blood cells (RBCs) are estimated to be rapid (Devoy et al. 2016). Because Cr(III) exhibits a lower rate of transport through cellular membranes than Cr(VI), Cr(III) remains trapped in RBCs. The RBC to plasma ratio has been used to indirectly infer cellular uptake and partitioning (and hence distribution and absorption), although this becomes unreliable if ratios exceed 1 as may occur following high acute or chronic exposure (Kirman et al. 2013). Only total chromium can be reliably

measured in tissues. In evaluating dose-response relationships for chromium, uncertainty related to tissue speciation needs to be explicitly considered.

At the most refined, information-rich level, dose-response analysis describes the relationship between external exposure and active chemical form at the target tissue and the response of concern. As noted above, NTP (2008) conducted a 2-year lifetime rodent studies, and Cr(VI) administered in drinking water induced oral cavity tumors in rats and small intestinal tumors in mice. Cr(III) is an essential element. It is noteworthy that tumors most strongly associated with Cr(VI) exposure originate relatively near sites of entry, i.e. lung in humans, oral cavity in rats and small intestine in mice. For this reason, understanding and quantifying the reduction of Cr(VI) in the oral cavity, stomach and small intestine is critically important for reliable interspecies extrapolation of rodent findings to humans (Schlosser and Sasso 2014).

The ability to evaluate the relationship between external exposure and internal dose is uncertain for Cr because analytical technology available to speciate the metal is limiting. In the case of chromium, only total chromium (the sum of all present valence states) can be reliably measured in tissues, where as Cr(VI) and Cr(III) can be reliably speciated in aqueous systems. Cr(VI) membrane transport is carrier-mediated, whereas Cr(III) transport is via diffusion. Based on differences in cellular uptake and partitioning, speciation (and hence distribution and absorption) can be indirectly inferred based on red blood cell to plasma ratio, although this becomes unreliable if ratios exceed 1 (Kirman et al. 2013). In evaluating dose-response relationships for chromium, uncertainty related to speciation needs to be explicitly considered limited. In the presence of uncertainty concerning target tissue concentration of Cr(VI), it is health protective to assume that the entire amount reaching the target tissue/organ is in the more toxic Cr(VI) toxic form associated with the dichromate compound exposures. ~~If incorrect, this will have the effect of overestimating dose to target tissue and hence risk. This would be the operative assumption if dose-response analysis is conducted using administered dose (e.g. concentration in drinking water) rather than dose of Cr(VI) reaching the target tissue.~~

In the spectrum of dose-response analysis, use of a physiologically-based pharmacokinetic (PBPK) model is the most information rich and scientifically sound basis for animal to human extrapolation. In the case of Cr(VI), rodent and human PBPK models are available that are based upon a large body of mechanistic pharmacokinetic data published in the peer-reviewed scientific literature (e.g., Thompson et al. 2011b; Kirman et al. 2012, 2013, 2017). Use of a PBPK model for dose-response assessment in support of health-protective exposure limit development is most reliably accomplished through an independent review and evaluation of all aspects of the model, including: source and reliability of physiological and chemical-specific parameters, assumptions regarding tissue transport, distribution and partitioning, adequacy of model evaluation, and impact of parameter variability and uncertainty (McLanahan et al. 2012).

Multiple analyses have utilized PBPK-models integrated into a mode of action framework to derive safe exposure levels for human populations (e.g., Thompson et al. 2013, 2014, 2018). Acceptance of these exposure limits for use in human health risk assessment has two basic requirements - acceptance of both the PBPK model and assumed mode of action as reliable and scientifically defensible. The next sections review the complex evidence supporting multiple modes of action for induction of carcinogenicity for Cr(VI).

Evidence for a mutagenic mode of action, which favors a linear approach

This section considers the mode of action evidence on the mutagenic potential of Cr(VI) by oral exposures. In the absence of information to the contrary, a conclusion that Cr(VI) may act via a mutagenic mode of action supports the use of a linear, no-threshold dose-response relationship in a cancer risk assessment.

As described in the USEPA Guidelines for Carcinogen Risk Assessment (USEPA 2005), understanding the mode of action is relevant to estimating cancer risk:

> "Determination of carcinogens that are operating by a mutagenic mode of action, for example, entails evaluation of in vivo or in vitro short-term testing results for genetic endpoints, metabolic profiles, physicochemical properties, and structure-activity relationship (SAR) analyses in a weight-of-evidence approach (Dearfield et al. 1991;

> U.S. EPA, 1986b; Waters et al. 1999). Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA. Also, mutagenic carcinogens usually produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed in vivo which generally are supported by positive tests in vitro." USEPA Guidelines pp 2-30.

A description and interpretation of various assays that provide information on the potential for a mutagenic mode of action conclusion are provided in USEPA (2005) and in the USEPA Guidelines for Mutagenicity Risk Assessment (USEPA 1986).

Evaluation of evidence

Evidence for the mutagenicity[1] of Cr(VI) is extensive and complex. The evidence to be considered includes the following:

> Mutagenic endpoints "include point mutations (i.e., submicroscopic changes in the base sequence of DNA) and structural or numerical chromosome aberrations. Structural aberrations include deficiencies, duplications, insertions, inversions, and translocations, whereas numerical aberrations are gains or losses of whole chromosomes (e.g., trisomy, monosomy) or sets of chromosomes (haploidy, polyploidy). Certain mutagens, such as alkylating agents, can directly induce alterations in the DNA. Mutagenic effects may also come about through mechanisms other than chemical alterations of DNA ~~("epigenetic[2] modifications")~~. Among these are interference with normal DNA synthesis (as caused by some metal mutagens), interference with DNA repair, abnormal DNA methylation, abnormal nuclear division processes, or lesions in non-DNA targets (e.g., protamine, tubulin)." (USEPA Guidelines for Mutagenicity Risk Assessment pp 4).

[1] A mutation is a heritable change in the DNA sequence, a common early event in tumor development.

~~[2] Epigenetic changes are functionally relevant and heritable changes to DNA that do not involve direct alteration of the DNA (nucleotide) sequence. Epigenetic changes may change how DNA is expressed or alter gene activity.~~

> "In evaluating chemicals for mutagenic activity, a number of factors will be considered: (1) genetic endpoints (e.g., gene mutations, structural or numerical chromosomal aberrations) detected by the test systems, (2) sensitivity and predictive value of the test systems for various classes of chemical compounds, (3) number of different test systems used for detecting each genetic endpoint, (4) consistency of the results obtained in different test systems and different species, (5) aspects of the dose-response relationship, and (6) whether the tests are conducted in accordance with appropriate test protocols agreed upon by experts in the field." USEPA Guidelines for Mutagenicity Risk Assessment pp 8).

Results from laboratory animal studies are judged to be informative as indicated by USEPA (1986):

> Despite species differences in metabolism, DNA repair, and other physiological processes affecting chemical mutagenesis, the virtual universality of DNA as the genetic material and of the genetic code provides a rationale for using various nonhuman test systems to predict the intrinsic mutagenicity of test chemicals. Additional support for the use of nonhuman systems is provided by the observation that chemicals causing genetic effects in one species or test system frequently cause similar effects in other species or systems.

Potentially relevant studies evaluating Cr(VI) mutagenicity include exposures via drinking water, oral gavage, intratracheal instillation and intraperitoneal (i.p.) injection, and in vitro mutagenicity studies. The drinking water and oral gavage studies are clearly relevant to the SSAB charge to recommend the appropriate science to be used for development of regulatory standards protective of public health and the environment for groundwater and surface water. Unfortunately, the database of drinking water studies is very limited. The intratracheal and i.p. studies also are potentially informative though interpretation of results from these studies is more complex due the differing absorption, distribution, metabolism and excretion (ADME) of Cr(VI) via these routes. The laboratory studies available are summarized below. Human studies are limited to exposures via inhalation and are briefly identified below. Differences in

ADME are an important consideration in interpreting the relevance of results from these inhalation studies to drinking water risk assessment.

Oral exposures via drinking water

Three studies (O'Brien et al. 2013; Thompson et al. 2015a; Aoki et al. 2019) have been published that specifically looked for increased mutation frequency in tumor target tissues in rodents. Sodium dichromate dehydrate exposed B6C3F$_1$ mice (0.3–520 mg/L in drinking water for 7 and 90 days) showed no increased K-*Ras*[3] codon 12 GAT mutations in duodenum (O'Brien et al. 2013). Exposure of Big Blue® TgF344 rats to 180 mg/L Cr(VI) in drinking water for 28 days did not significantly increase the mutant frequency in the *cII* transgene in the gingival/buccal or the gingival/palate regions relative to controls (Thompson et al. 2015a). Sodium dichromate dihydrate was administered orally in drinking water to male *gpt* delta mice at a dose of 85.7 or 257.4 mg/L for 28 days or at a dose of 8.6, 28.6 or 85.7 mg/L for 90 days; no significant increase in *gpt* mutant frequency relative to that in control mice was observed in the small intestine (Aoki et al. 2019). Two of the studies (Thompson et al. 2015a and Aoki et al. 2019) were conducted in transgenic (genetically modified) rodents (Big Blue® rats and *gpt* delta transgenic mice); these systems can detect point mutations and small-scale deletions but are not sensitive to larger deletions or aneuploidy (gain or loss of whole chromosomes). The O'Brien et al. (2013) study (in mice) only looked for mutations at K-*Ras* codon 12. Codon 12 is one of several codons in K-*Ras* that have been implicated in human colon cancers, and K-*Ras* is one of several oncogenes[4] known to be mutated in human colon cancer.

The results of micronuclei from rodent drinking water studies are mixed positive and negative (Mirsalis et al. 1996; De Flora et al. 2006; NTP 2007; O'Brien et al. 2013; Thompson et al. 2015b). Mirsalis et al. (1996) reported no statistically significant increase in micronucleated RNA-positive erythrocytes in mice allowed ad libitum access to drinking water with up to 20 mg/L Cr(V1) for 48 hr. De Flora et al. (2006) reported no increase of the micronucleus

[3] *Ras* genes are involved normal cell growth regulation and differentiation pathways. Alterations of *ras* genes can change their ability to function properly, potentially resulting in sustained cell growth and proliferation, a major step in the development of cancer.

[4] On oncogene is a gene with the potential to cause cancer.

frequency in bone marrow or peripheral blood erythrocytes of mice exposed to sodium dichromate dihydrate and potassium dichromate administered with drinking water up to a concentration of 500 mg/L Cr(VI) for up to 210 days. NTP (2007) summarize two studies and concluded the "... results of four micronucleus tests conducted in three strains of mice were mixed." In study 1, male and female B6C3F$_1$ mice were given drinking water containing up to 1,000 mg sodium dichromate dihydrate/L for 3 months. No significant increases were seen in micronucleated normochromatic erythrocytes in peripheral blood samples. In study 2, micronucleus frequencies were evaluated in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice administered sodium dichromate dihydrate up to 250 mg/L in drinking water for 3 months. A significant exposure concentration-related increase in micronucleated normochromatic erythrocytes was seen in *am3*-C57BL/6 male mice (in two of the three exposed groups of this strain, micronuclei were significantly elevated). An increase in micronucleated erythrocytes was noted in male B6C3F$_1$ mice but judged by the authors to be "equivocal" based on a small increase in micronuclei of exposed groups that did not reach statistical significance above the control group. No increase in micronucleated normochromatic erythrocytes was observed in male BALB/c mice (NTP 2007). No exposure-related effects on the percentage of polychromatic erythrocytes was observed in any of the three mouse strains tested. Concerns include that these results were mixed; the only positive findings were sex- and strain-specific in *am*3-c57BL/6 male mice with results judged "equivocal" in the B6C3F$_1$ mouse strain that has typically been used for NTP carcinogenicity testing.

O'Brien et al. (2013) report that sodium dichromate dehydrate exposed B6C3F$_1$ mice (0.3–520 mg/L in drinking water for 7 and 90 days) showed no increased micronuclei and karyorrhectic nuclei in the duodenal crypts. Thompson et al. (2015b) report Cr(VI), in the form of sodium dichromate dehydrate in drinking water up to 180 ppm for 7 days, did not increase micronuclei in female B6C3F$_1$ mice.

Other endpoints from Cr(VI) exposures via drinking water include DNA deletions which were positive (Kirpnick-Sobol et al. 2006). Pregnant C57BL/6Jp^{un}/p^{un} mice were given free access to Cr-supplemented drinking water (potassium dichromate used at 62.5 or 125.0 mg/L, and 20-day-old offspring were harvested to examine for DNA deletions. In this model, a

somatic deletion reconstitutes the wild-type p gene, resulting in black-pigmented cells (eyespots) on the retinal pigment epithelium. Offspring of mice treated with Cr(VI) had statistically-significant increases in the number of eyespots on the retinal epithelium, that study's measure of the frequency of DNA deletions. The background (control) eyespot frequency was significantly increased by 27% and 38% in the treated groups, respectively, although the treated group frequencies were not significantly different from one another. Concerns include that exposures of embryos was transplacental during a highly sensitive 10 day period in their development (the mother received Cr(VI) via drinking water, but the assay was of the offspring). Also, there was no significant dose-response in the treated groups, sample sizes of the treated groups were markedly lower (n=24 and 14) versus the n=55 for the control group (this discrepancy in sample sizes is not explained and could be a source of bias), and a scan of PubMed failed to reveal other studies that have replicated this finding.

In other Cr(VI) drinking water studies, DNA double-strand breaks are negative (Thompson et. al. 2015c; Sánchez-Martín et al. 2015); DNA protein cross-links are negative (De Flora et al. 2008; Coogan et al. 1991); increased complexing of proteins with DNA was demonstrated in liver following 3 weeks of exposure at both 100 and 200 ppm chromium (Coogan et al. 1991), and unscheduled DNA synthesis was negative (Mirsalis et al. 1996).

The negative mutation frequency studies coupled with the mixed positive and negative results from the micronuclei and DNA studies make the interpretation complex. Overall, these studies provide suggestive evidence that Cr(VI) drinking water studies may produce mutations relevant to a mutagenic mode of action for carcinogenesis.

Oral exposures via gavage

Similarly, the rodent gavage studies are mixed with positive and negative results. Three micronuclei studies in mice have been published, all with negative results (Shindo et al. 1989; Mirsalis et al. 1996; De Flora et al. 2006). Three studies in mice of DNA damage using the comet assay have been published, all indicating positive results (Dana Devi et al. 2001; Sekihashi et al. 2001; Wang et al. 2006).

These studies provide suggestive evidence that exposure by gavage to Cr(VI) may produce mutations relevant to a mutagenic mode of action for carcinogenesis, though interpretation of the comet assays is uncertain.

Intratracheal and inhalation exposures

Two studies by intratracheal exposures have shown positive results, one each for mutations in mice (Cheng et al. 2000) and DNA alterations in rats (Izzotti et al. 1998).

A single inhalation study in rats exposed to chromium fumes showed chromosomal aberrations and sister chromatid exchange in bone marrow and peripheral lymphocytes, but the valence state was not specified (Koshi et al. 1987).

These studies provide evidence that exposure by intratracheal instillation to Cr(VI) may produce mutations relevant to a mutagenic mode of action for carcinogenesis, though interpretation of the results is uncertain due to differences in ADME from drinking water or oral gavage studies.

Intraperitoneal exposures

At least 14 studies by multiple investigators have been published, all of which indicated positive results for mutation frequency, dominant lethal mutations, micronuclei, DNA damage via the comet assay, or suppressed nuclear DNA synthesis (Wild 1978; Knudsen 1980; Amlacher and Rudolph 1981; Hayashi et al. 1982; Paschin and Toropzev 1982; Paschin et al. 1982; Shindo et al. 1989; Itoh and Shimada 1996, 1997, 1998; Wronska-Nofer et al. 1999; Sekihashi et al. 2001; Ueno et al. 2001; De Flora et al. 2006).

These studies provide potential evidence that exposure by i.p. injection to Cr(VI) may produce mutations relevant to a mutagenic mode of action for carcinogenesis, though interpretation of the results is uncertain due to differences in ADME from drinking water or oral gavage studies.

Studies of specimens collected from humans

A large number of studies (many dozens) have been conducted on blood, buccal, urine and other samples with many showing positive results for chromosomal aberrations, micronucleus assay, sister chromatid exchange, DNA strand breaks, etc. The interpretation of these results as they relate to drinking water exposure is uncertain because the route of exposure in the subjects may be via drinking water, food, and/or inhalation. Nonetheless, the studies clearly show that Cr(VI) exposure results in positive test outcomes indicating a potential mutagenic mode-of-action.

Cytotoxic mode of action, which favors a non-linear approach

In certain circumstances, the 2005 USEPA Guidelines for Carcinogen Risk Assessment allow for a non-linear dose-response assessment as a plausible alternative to the default "linear through zero" assessment utilizing a linearized multi-stage model analysis of tumor incidence data. These circumstances include 1) significant evidence of a tumor response at only one or two of the highest doses in a cancer bioassay, with little or no evidence of a tumor response at the lower doses; 2) significant evidence of related cytotoxicity and enhanced restorative cell proliferation in the target tissues at the same highest doses and temporally preceding the tumor responses, again with little or no evidence of this precursor response at the lower doses; and 3) little or no evidence of *in vivo* genotoxicity in the target tissues. The most relevant lines of evidence for a non-mutagenic mode of actions are replicated aspects of the NTP's Cr(VI) drinking water studies with B6C3F$_1$ mice and F344 rats but adding lower doses relevant to environmental exposures. While of shorter duration than the NTP studies, sodium dichromate dehydrate exposed B6C3F$_1$ mice (0.3–520 mg/L in drinking water for 7 and 90 days) showed no increased K-*Ras* codon 12 GAT mutations in duodenum, micronuclei or karyorrhectic nuclei in the duodenal crypts (O'Brien et al. 2013). Exposure of Big Blue® TgF344 rats to 180 mg/L Cr(VI) in drinking water for 28 days did not significantly increase the mutant frequency in the *cII* transgene in the gingival/buccal or the gingival/palate regions relative to controls (Thompson et al. 2015a). Sodium dichromate dihydrate in drinking water to male *gpt* delta mice at a dose of 85.7 or 257.4 mg/L for 28 days or at a dose of 8.6, 28.6 or 85.7 mg/L for 90 days produced no significant increase in *gpt* mutant frequency in the small intestine (Aoki et al. 2019). The mechanism of action posited for a non-mutagenic

mechanism of action in the small intestine starts with unreduced Cr(VI) absorption into villus enterocytes (at doses exceeding the body's ability to reduce Cr(VI) to Cr(III)), cytotoxicity, compensatory hyperplasia, and increased cell replication which increases the chance of spontaneous mutations and carcinogenesis.

Physiologically-based pharmacokinetic modeling provides a useful adjunct to the tumor, cytotoxicity, and restorative cell proliferation data that can link these endpoints directly to predicted fluxes and/or concentrations of the presumptive toxic moieties in target tissues and provide scientific support for high-to-low dose and interspecies risk extrapolations.

The mechanistic toxicology database for Cr(VI) is extensive. Oral and intestinal tumor data are available for rats and mice, respectively, from well-conducted NTP drinking water studies. Data for diffuse epithelial hyperplasia, the precursor lesion associated with the mouse intestinal tumors are also available from the same NTP drinking water study. A PBPK model has been developed by Kirman et al. (2017) that predicts 1) pyloric flux of Cr(VI) from the stomach lumen to the lumen of the small intestine, 2) sectional tissue uptake of Cr(VI) from the small intestine lumen, and 3) Cr(VI) flux from small intestinal tissues to the portal plasma. The data are thus sufficient to estimate a lower bound Benchmark Dose and an associated RfD for both intestinal tumors and diffuse epithelial hyperplasia.

Comparative weight of evidence for potentially relevant modes of action

The evidence regarding the potential for a mutagenic mode of action for Cr(VI) oral exposures is complex and difficult to interpret, but evidence exists that indicates a mutagenic MOA may be operative which supports application of a linear dose-response assessment. Animal in vivo studies and studies of specimens from exposed humans comprise the evidence evaluated here. The results from drinking water and gavage studies are mixed. Mutation frequency studies are negative but uncertain due to gaps in the assays, whereas micronuclei and DNA aberration studies are mixed positive and negative with interpretation challenges due to the assays employed. The intratracheal and i.p. studies indicate Cr(VI) may cause mutations, but there is uncertainty about ADME and hence interpretation of results.

The data from human studies clearly show that Cr(VI) via inhalation can cause cancer ~~mutations~~ (Group A carcinogen) via mechanisms that include the induction of DNA damage among other genotoxic effects, with evidence that a mutagenic mode of action is potentially operative. There is a paucity of studies from human exposures to Cr(VI) via drinking water.

The case can be made for a non-linear dose-response assessment for Cr(VI) carcinogenicity as a plausible alternative to EPA's default "linear through zero" approach to the assessment of genotoxic carcinogens. Recent references for a cytotoxic mode of action identified using PubMed include Kopec et al. 2011; Proctor et al. 2011, 2012; Thompson et al. 2011a, b, 2012a-c, 2013, 2014, 2015a-c, 2016a, b, 2017a-c, 2018; O'Brien et al. 2013; Suh et al. 2014, 2019; Rager et al. 2017; and Aoki et al. 2019. The database is substantial and robust. It includes more than two dozen peer-reviewed publications that describe how a non-linear assessment was developed by acquiring extensive mechanistic data relevant to Cr(VI) carcinogenicity. A non-linear dose-response assessment merits serious consideration. Mutagenicity data for Cr(VI) in the oral mucosa and duodenum of Big Blue® rats exposed to Cr(VI) in drinking water are negative (Thompson et al. 2015a, 2017b). Furthermore, there were no dose-related increases in K-*Ras* mutant frequency, micronuclei formation, or change in mitotic or apoptotic indices in crypt tissues taken from mice exposed to Cr(VI) in drinking water (O'Brien et al. 2013) and no significant increase in *gpt* mutant frequency in small intestines of male *gpt* delta mice exposed to Cr(VI) in drinking water (Aoki et al. 2019). Gaps in knowledge affect the confidence in conclusions that can be drawn about a mutagenic (linear) mode of action and the potential for carcinogenesis from oral exposure to Cr(VI).

Differences among scientists on the interpretation of studies, and the potential importance of gaps in knowledge, result in debates as to the strength or weight of the evidence and the corresponding conclusions drawn. Risk assessors have an important role in conveying to decision makers the strength and uncertainties of the evidence and the conclusions drawn. Communication of complex scientific knowledge can be difficult. In the end, scientific judgment is necessary and expected:

> "Generally, "sufficient" support [regarding a carcinogenic mode of action] is a matter of scientific judgment in the context of the requirements of the decision maker or in the

context of science policy guidance regarding a certain mode of action." USEPA Guidelines pp 2-42

Summary and Recommendations

1) A decision to select a linear no-threshold approach or a non-linear dose-response approach for oral exposures to hexavalent chromium (Cr(VI)) is informed by consideration of the toxicological and epidemiological evidence, particularly as it informs mode of action. A mutagenic mode of action in carcinogenesis would typically lead to assumption of a linear no-threshold approach to dose-response assessment (resulting in an estimate of an oral slope factor, OSF) whereas a non-mutagenic mode of action (e.g., effects due to cytotoxicity) would typically lead to assumption of a non-linear approach based on identification of a point of departure and application of uncertainty factors (resulting in an estimate of a reference dose, RfD). At low doses a mutagenic mode of action may be operative whereas at higher doses cytotoxicity or other mechanisms may be operative. Therefore both mutagenic and cytotoxic modes of action may result from chemical exposure with mutagenicity occurring at all levels of exposure and as the putative mode of action in the low-dose region. We derived recommendations following the USEPA's Guidelines for Carcinogen Risk Assessment (USEPA 2005) and Guidelines for Mutagenicity Risk Assessment (USEPA 1986).

2) Given currently available evidence, the State should base health protective goals on the highest quality lifetime studies in rodents (e.g., National Toxicology Program bioassays) and place the greatest emphasis on studies of rodent tumor responses and the mode of action by which these adverse effects developed. Particularly important are mechanistic studies in similar human tissues along with associated pharmacokinetics information to help with cross-species extrapolation. As cancer endpoints drive a recommendation for Cr(VI), the focus should be on the relevant cancer mode of action studies. Authoritative reviews (e.g., by ATSDR, EPA IRIS, or CalEPA) may be useful references.

3) The data from human studies clearly show that Cr(VI) exposure via inhalation can cause ~~mutations and~~ cancer via mechanisms that include the induction of DNA damage among other genotoxic effects, with evidence that a mutagenic mode of action is potentially operative. In 2-year lifetime rodent studies, NTP concluded that there was clear evidence of carcinogenic activity of Cr(VI) exposure via drinking water based on observations of

increased incidences of oral cavity tumors in male and female rats, and small intestinal tumors in male and female mice. The evidence regarding the potential for a mutagenic mode of action for Cr(VI) oral exposures is complex and difficult to interpret with positive and negative findings and interpretation challenges due to the assays employed. The available drinking water mutation frequency studies are negative. The results from drinking water studies of micronuclei are mixed positive and negative; DNA deletions are positive; DNA double-strand breaks are negative; DNA protein cross-links are mixed; and unscheduled DNA synthesis are negative. Similarly, the rodent gavage studies are mixed with negative results in micronuclei and positive findings studies of DNA damage using the comet assay. The available intratracheal and intraperitoneal studies indicate Cr(VI) may cause mutations, but there is uncertainty about absorption, distribution, metabolism and excretion of Cr(VI) via these routes and hence interpretation of results.

4) Data published between 2005 and 2019 from drinking water studies with rats and mice have been the subject of robust mechanistic toxicity assessments of cancers in the oral cavity and intestine. Available mutagenicity studies conducted during this period were negative; there were not dose-related increases in K-*Ras* mutant frequency or change in mitotic or apoptotic indices, and micronuclei formation was negative in six of seven studies over the time period. Toxicant localization and histological examinations have helped elucidate the mode of action in the rodent drinking water studies. If considering the mouse and rat drinking water exposure studies only, there is strong support for a non-mutagenic mode of action for intestinal tumors involving chronic wounding of intestinal villi and crypt cell hyperplasia. This was the basis of Health Canada and Food Safety Commission of Japan conclusions which placed more emphasis on oral exposures and mode of action studies most relevant to the critical effect endpoint and less emphasis on other endpoints or routes of exposure. Importantly, rat oral tumors were not preceded by hyperplasia, and results demonstrating wounding of intestinal villi and crypt cell hyperplasia do not account for these tumors (but a transgenic rodent mutation assay in the oral cavity of Big Blue® F344 rats was negative for mutation).

5) The mixed positive and negative genotoxicity results from laboratory studies via non-inhalation exposure routes, coupled with clear evidence in humans that Cr(VI) exposure via inhalation damages DNA and is ~~mutagenic and~~ carcinogenic, provide evidence that a

mutagenic mode of action is potentially operative for Cr(VI) exposures via drinking water. However there is only very limited evidence from Cr(VI) drinking water studies of a mutagenic mode of action.

6) Multiple modes of action may be occurring simultaneously and the sequence of events leading to cancer formation is uncertain. Significant data gaps and uncertainties remain (e.g., mode of action assessment in the few rodent drinking water studies address a limited suite of endpoints, and there is evidence of mutagenic responses in tissues other than where tumors occur). There is not conclusive evidence to rule out a mutagenic mode of action, and we conclude that Cr(VI) via drinking water exposure may cause mutational changes. Further, remaining uncertainties (e.g., physiologically-based pharmacokinetic modeling) are such that we could not definitively choose among the modes of action, and therefore quantitative dose response assessment leading to both an OSF and RfD should be explored by the State. Due to the remaining uncertainty and because it is generally considered to be a more health-protective approach, the SSAB recommends the State consider a linear extrapolation approach ~~As a science guided policy, the SSAB recommends the State consider a linear extrapolation approach because of the remaining uncertainty and because it generally is considered to be a more health-protective approach~~ (this was a majority view; one member thought no science-guided policy recommendations should be offered).

7) The SSAB recommends that State risk assessment staff closely monitor the USEPA's IRIS update of Cr(VI) toxicity. The USEPA's data synthesis and review is going on now; a contemporary review of that magnitude is extremely valuable for further refinement of mode of action recommendations. According to the ~~most recent~~ October 2020 IRIS timeline (https://www.epa.gov/iris/iris-program-outlook), the target date for the Cr(VI) Public Comment Draft is ~~spring~~ summer 2021.

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Comments received on February 2020 draft Secretaries' Science Advisory Board *Memorandum on Response to Inquiry on Hexavalent Chromium*

Commenter(s)	Comments made (pulled from original PDFs, already	SSAB draft notes / preliminary responses	Disposition in proposed final version
C. Thomas Alley Jr., Vice President, Generation, Electric Power Research Institute 5/28/2020	1) The SSAB's recommendation largely rests on: (1) the finding that Cr(VI) causes lung cancer in workers by a mutagenic mode of action (MOA); (2) the "mixed" genotoxicity and mutagenicity assay results in the peer-reviewed literature and the National Toxicology Program (NTP) data; and (3) the potential existence of multiple modes of action wherein mutagenicity occurs at all doses, and cytotoxicity occurs only at high doses.	Perspective. Opportunity to clarify the various lines of evidence evaluated by the SSAB, but those are already discussed in the document.	No changes needed.
	2) Several governmental agencies and scientific organizations (IARC, 2012; ATSDR, 2012; TCEQ, 2014) have indicated that the MOA for Cr(VI)-induced lung cancers is expected to include non-mutagenic mechanisms such as oxidative stress and inflammation, deregulation of mismatch repair genes, and genomic instability, or that the evidence for genotoxicity is limited. Further, while the SSAB cites IARC (2012) as support for the assertion that Cr(VI) is mutagenic via inhalation exposure, IARC does not offer a conclusion regarding the MOA for lung tumors.	Dr. DeWitt revisited each of the references mentioned in this comments and relayed the following: 1) IARC monograph 100C (2012). Here is the language from the monograph: "Several mechanisms are involved in the carcinogenesis induced by chromium (VI) that include the induction of DNA damage, the generation of oxidative stress and aneuploidy, leading to cell transformation. With respect to DNA damage, the spectrum of induced lesions appears to depend strongly on the cellular reductant involved. Thus, under physiological conditions with ascorbate as the major reductant, the generation of premutagenic ternary chromium–ascorbate–DNA adducts appears to be of major relevance, which may be linked to the increased number of mismatch-repair-resistant cells observed in chromate-induced lung tumours." The SSAB citation appears to be appropriate with the IARC synthesis statement for carcinogenic mechanisms. 2) ATSDR, 2012. Here is the language on genotoxicity from the ATSDR: "Numerous studies have evaluated the genotoxicity of chromium(VI) compounds. Results of occupational exposure studies in humans, although somewhat compromised by concomitant exposures to other potential genotoxic compounds, provide evidence of chromium(VI)-induced DNA strand breaks, chromosome aberrations, increased sister chromatid exchange, unscheduled DNA synthesis, and DNA-protein crosslinks. Although most of the older occupational exposure studies gave negative or equivocal results, more recent studies have identified chromosomal effects in exposed workers. Findings from occupational exposure studies are supported by results of in vivo studies in animals, in vitro studies in human cell lines, mammalian cells, yeast and bacteria, and studies in cell-free systems." 3) TCEQ, 2014. Texas had this to say about nonlinear approaches: "However, whether data relevant to the carcinogenic MOA and epidemiological analyses support consideration of nonlinear-threshold assessments for CrVI inhalation carcinogenicity is subject to scientific debate, and the uncertainties associated with the assessment (e.g., limited statistical power of epidemiological studies to detect increased risk at low exposure levels, lack of a statistically better fitting threshold model, lack of data on competing rates of extracellular CrVI reduction and lung tissue absorption) appear to preclude a robust scientific justification for deviation from the default linear low-dose extrapolation approach. Thus, the nonlinear-threshold assessment is not a focus of this document and the default linear low-dose extrapolation approach is utilized in the following sections to derive URF estimates based on various epidemiological studies." [note: That is the conclusion from TCEQ's 2014 technical support document on particulate forms of hexavalent chromium, which is cited by this commenter. We note that TCEQ also has a 2016 technical support document on hexavalent chromium oral reference dose which concludes that cytotoxicity-induced regenerative hyperplasia is the most scientifically supported mechanism of carcinogenesis by the oral route and that a non-linear, point of departure based reference dose be used. The SSAB discussed TCEQ's approach with one of their senior scientists who presented to the SSAB]. Dr. Vandenberg notes that it was not necessary for IARC to make a conclusion regarding MOA; there was sufficient evidence of cancer in human and animal evidence for an overall evaluation of carcinogenicity to humans (Group 1). See Table 4 of the IARC evaluation framework (https://monographs.iarc.fr/wp-content/uploads/2019/07/Preamble-2019.pdf). The IARC synthesis of their review of mechanistic information was brief but clearly acknowledges the role of DNA damage in lung cancer: "Several mechanisms are involved in the carcinogenesis induced by chromium (VI) that include the induction of DNA damage, the generation of oxidative stress and aneuploidy,..." (entire quotation from the IARC synthesis is provided above)	The SSAB citation appears to be appropriate with the IARC synthesis statement for carcinogenic mechanisms. However the commenter's point is taken that the reference does not use the term mutagenesis but rather lists evidence of Cr(VI)-induced DNA damage. We have rephrased references to mode of action in inhalation studies to include the induction of DNA damage among other genotoxic effects with evidence that a mutagenic mode of action is potentially operative.
	3) Recent robust studies on lung cancer MOA related to inhalation exposures have been published (Procter et al., 2014; Rager et al., 2019). These studies provide evidence that supports a non-mutagenic MOA for Cr(VI)-induced lung cancer and molecular events related to epigenetic mechanisms.	Context. SSAB analysis considered but did not rely heavily the mechanism of action for inhalation exposures. SSAB's review considered and referenced evidence and perspective for a mutagenic and non-mutagenic mutagenic mechanism of action.	No changes needed.

	4) SSAB review could be improved by focusing on the high quality target tissue mutagenicity and genotoxicity data from drinking-water exposures—the only relevant pathway of exposure for this review. There is uncertainty around data from other routes of exposure, data from non-standardized protocols, and data from non-target tissues. The high-quality target tissue mechanistic data that exist in the peer-reviewed scientific literature strongly and consistently support a non-mutagenic MOA.	Perspective. Dr. Vandenberg notes evidence from other routes of exposure are potentially relevant unless evidence indicates otherwise. SSAB checked on how we referenced and characterized intraperitoneal and intratracheal exposures. Our draft noted that intratracheal and intraperitoneal studies indicate Cr(VI) may cause mutations, but there is uncertainty about absorption, distribution, metabolism and excretion of Cr(VI) via these routes and hence interpretation of results (i.e., context for these observations was provided in the draft).	No changes needed.
	5) It would be helpful for the SSAB to provide evidence to support its assertion that there may be multiple MOAs wherein Cr(VI) is mutagenic at all exposures and cytotoxic only at high exposures. This is particularly true given that there are no incidence data for low-dose tumors in small intestinal tissue.	Context (opportunity to provide additional context)	Review of meeting minutes and discussions revealed no additional context was available on this point.
	6) The SSAB has indicated that "Cr(VI) is a recognized human carcinogen with mutagenic action in inhalation exposure" (p. 4, lines 116-117) and cites IARC (2012) to support that statement. However, EPRI notes that IARC does not offer a conclusion regarding the MOA for lung tumors.	See notes above in response to Comment #2 from this commenter.	See notes for comment 2 above.
	7) The SSAB indicated that epigenetic modifications are considered equivalent to mutagenicity by citing the EPA (1986) guidance on mutagenicity risk assessment (page 10 of SSAB document); however, the document referenced is outdated, and the scientific community now differentiates a mutagenic MOA from an epigenetic MOA (e.g., Preston, 2007).	Dr. DeWitt conveyed that the most current Guidelines for Mutagenicity Risk Assessment were published in 1986, so what is referenced is current. Preston, 2007 is an article written by a single author (Julian Preston) who used to lead one of the labs at the US EPA. This one article is in no way representative of the "scientific community." Preston, 2007 concerns the revised Guidelines for Carcinogen Risk Assessment (which were revised by the US EPA in 2005) and does address epigenetic modifications with respect to carcinogenesis but not to mutagenesis. Dr. Dorman indicated there was no need to link these instead of treating as separate lines of evidence. Dr. Augspurger notes that the parenthetical, ("epigenetic modifications"), is not part of the 1986 reference which was being quoted directly in the draft.	The parenthetical, ("epigenetic modifications") and its associated footnote, were removed from what is intended to be a direct quote from the subject reference.
	8) On page 17 of the SSAB memorandum, the SSAB states that "human studies clearly show that Cr(VI) via inhalation can cause mutations." This statement appears to be inconsistent with ATSDR.	Dr. DeWitt relays what the ATSDR (2012) writes about inhalation and mutations: "Thus, the available studies support that chromium compounds, particularly chromium(VI), have carcinogenic potential because interactions with DNA have been linked with the mechanism of carcinogenicity." Figure 3-8 of the ATSDR document indicates genotoxicity from inhalation exposure is a human health effect of Cr(VI). It should also be noted that the majority of occupational studies addressed in the ATSDR profile concern inhalation exposures. Also, ATSDR is not cited here by SSAB.	The SSAB citation appears to be appropriate with ATSDR synthesis statement for carcinogenic mechanisms. However the commenter's point is taken that the reference does not use the term mutagenesis but rather lists evidence of Cr(VI)-induced DNA damage. We have rephrased references to mode of action in inhalation studies to include the induction of DNA damage among other genotoxic effects with evidence that a mutagenic mode of action is potentially operative.
	9) ... TCEQ used data from Painesville and Baltimore chromate production workers (Crump et al., 2003; Gibb et al., 2000; Luippold et al., 2003). TCEQ also examined the toxicology and kinetics of Cr(VI) and concluded that the evidence was not sufficient to conclude that Cr(VI) acts by a mutagenic MOA (Haney et al., 2012; TCEQ 2014). Notably, TCEQ indicated that the exposure-response relationship for lung cancer may be nonlinear, based on reduction of Cr(VI) to Cr(III) prior to absorption.	Perspective. TCEQ ultimately decided on a linear analysis for particulate chromium, noting "...a complete and clear picture of the MOA(s) for CrVI-induced lung carcinogenesis is yet to be elucidated and no MOA has been widely accepted by the scientific community as definitive."	No changes needed.

10) ... the SSAB document contains some statements regarding the MOA for occupational Cr(VI) induced lung cancer that are not consistent with several scientific bodies, including IARC.	This comment has been addressed in consistency checks of other comments by this commenter. See #2 and #8 above.	See notes for comments 2 and 8 above.
11a) Proctor et al. (2014) shows… In Cr(VI) industries where workers had elevated lung cancer risk, exposures to Cr(VI) were sufficiently high to cause respiratory tissue damage, such as ulcerated and perforated nasal septum (Miller, 1953; NIOSH, 1975; Sorahan et al. 1987; IARC, 1990; Gibb et al., 2000a, b; Luippold et al., 2003; Birk et al., 2006). Although low-dose linear models have been applied to the worker epidemiological data, there is no evidence specifically supporting low-dose linearity from the epidemiologic literature.	Perspective. Dr. Vandenberg suggests no change needed. The MOA is still relevant, but it does not seem necessary for the SSAB to discuss lung cancer epidemiology studies for an review focused on ground water/drinking water exposures. The comments seems to be trying to make arguments that the incidence of lung tumors in humans and animals only supports a non-mutagenic MOA but it is not clear why a mutagenic MOA could not also be operant. Gibb has very recently (July of this year) published a new analysis of the Baltimore cohort data, focusing on the effects of age and smoking: Gibb et al. (in press). The effect of age on the relative risk of lung cancer mortality in a cohort of chromium production workers. American Journal of Industrial Medicine. https://doi.org/10.1002/ajim.23152. Epidemiological exposures were however not a significant foundation of the SSAB's recommendations which focused on oral exposures.	No changes needed.
11b) Proctor et al. (2014) shows... Animal studies show that lung carcinogenicity is associated with tissue damage and inflammation induced by high-dose Cr(VI) exposure of bronchial tissues or microenvironments within the lung (Levy et al., 1986; Steinhoff et al., 1986; Glaser et al., 1990; Beaver et al., 2009). Glaser et al. (1986) exposed male Wistar rats for 18 months (22 hrs/day, 7 days per week) to submicron aerosols of sodium dichromate and pyrolyzed Cr(VI):Cr(III) oxide mixture (3:2). The animals were exposed to Cr(VI) at concentrations up to 100 µg/m3. Lung tumors were observed only at the highest doses and only in the presence of inflammatory response. The authors described the carcinogenic potency as “weak.”	Perspective. See response to comment #11a above.	No changes needed.

	11c) Proctor et al. (2014) shows… Observations from animal studies are consistent with the toxic kinetic data for Cr(VI); specifically, extracellular reduction of Cr(VI) to Cr(III) limits intracellular absorption of Cr(VI) and Cr(VI)-induced toxicity. However, this process can be overwhelmed at high exposure conditions (De Flora et al., 1997; Proctor et al., 2014). Data from Steinhoff et al. (1986) provide evidence for a dose-rate effect where cancer is induced at high exposures sufficient to overwhelm natural biological defenses. In this intratracheal instillation study, sodium dichromate or calcium chromate was administered to Sprague Dawley rats at dose rates of once per week or once per day (five times per week) to achieve weekly doses of 0.05, 0.25, or 1.25 mg/kg. A dose-rate effect was observed for both sodium chromate and calcium chromate at 1.25 mg/kg per week. In short, high doses administered once per week were more potent than the equivalent dose administered daily. Calcium chromate, which has a longer half-life in the lung, was more potent than sodium dichromate. Tumor formation was accompanied by irritation and inflammation; the authors concluded that irritation and inflammation are more important in tumor formation than dose.	Perspective. SSAB's review considered and referenced evidence and perspective for a non-mutagenic mutagenic mechanism of action. Presentations we received on a threshold, non-linear approach (e.g., Texas Commission on Environmental Quality, Health Canada, and ToxStrategies) and references describing a cytotoxic mechanism of action and resultant non-linear approach to reference dose derivation (e.g., pages 16 and 17) are cited in the draft.	No changes needed.
	11d) Proctor et al. (2014) shows… There is also human epidemiological evidence of a dose-rate effect. The Gibb et al. (2011) study of the Baltimore chromate production workers reported evidence of a dose-rate effect for lung cancer. ... Gibb et al. 2011) shows that, given the same cumulative exposure of 0.339 mg/m3-years Cr(VI), the relative risk for lung cancer mortality is greatest for both smokers and nonsmokers with short periods of exposure compared to longer durations of exposure. Gibb et al. concluded, “The same cumulative exposure over a short period of time (30 days) had more effect than if the exposure occurred over 10 years.”	Perspective. See response to comment #11a above.	No changes needed.
	12) The totality of evidence supports a nonmutagenic MOA for Cr(VI)-induced lung cancer and use of nonlinear approaches when extrapolating lung cancer risk at high-concentration occupational exposures to exposures in the environment (Proctor et al., 2014)	Perspective. See response to comment #11a above.	No changes needed.

13) At the mechanistic level, events linking Cr(VI) exposure to lung cancer have been proposed to include both genomic instability and epigenetic modifications (Browning et al., 2017; Holmes et al., 2008). However, precisely how these mechanistic events relate to the overall MOA has yet to be established. Further research is needed to substantiate these mechanisms, elucidate which molecular mediators are involved in carcinogenesis, and relate mechanistic events to the overall MOA for Cr(VI)-induced lung cancer.	Perspective. SSAB's review notes evidence for different mechanisms of action, remaining uncertainties, and differing opinions within the scientific community. These issues are reflected in the body of the document and its concluding recommendations.	No changes needed.
14) ... Rager et al. (2019) toxicogenomic analysis supports the influence of epigenetic alterations on cell signaling related to Cr(VI)-induced cytotoxicity and/or cell proliferation, and decreases in DNA repair signaling that lead to tumorigenesis.	Perspective and additional detail.	No changes needed.
15) NTP (2007) reports blood micronucleus (MN) assays from four experiments, all drinking water exposures. Two were negative, and a third was equivocal (i.e., lacked statistical significance or a dose response relationship). Only one study was positive, which consisted of data from a transgenic mouse strain (am3-C57BL/6), for which MN studies have not been reported for any other agent by NTP or other researchers. The SSAB refers to the results from these studies as “mixed” (Page 12, lines 358–381); however, the only reliable data from this report are negative and equivocal. No reproducible positive data are included in this dataset.	Dr. DeWitt researched this comments and notes the NTP (2007) report states: "The results of four micronucleus tests conducted in three strains of mice were mixed." Page 57 of the NTP report. Dr. DiGiulio recommends we use this direct quote from the NTP report (and cite it) to make it clear the authors of the study draw the conclusion we referenced in our draft recommendations.	Change suggested by Dr. DiGiulio made in proposed final version.
16) The SSAB states that the three published gavage MN studies were all negative, whereas three Comet assays were positive (Page 14, lines 418–426). The Board then concludes that the results from gavage studies are “mixed,” and that those results provide “suggestive evidence that exposure by gavage to Cr(VI) may produce mutations relevant to a mutagenic mode of action for carcinogenesis, though interpretation of the comet assays is uncertain.” It should be noted that the Comet assay is not a marker of mutation at the gene or chromosomal level; thus, the statement that the gavage data are mixed for mutagenicity requires clarification. Furthermore, gavage administration is not likely to be representative of drinking water exposure because high concentrations of Cr(VI) are delivered in a small volume bolus dose, which is more likely to overwhelm reduction of Cr(VI) to Cr(III), as compared to the same dose by drinking water administration.	Dr. DeWitt relays that the Comet assay is a measure of DNA damage in eukaryotic cells (it detects strand breaks in DNA). While technically a mutation is defined as a heritable change in the DNA sequence, the Comet assay is used for mutagenicity testing. This seems a very fine distinction that could be clarified but may be unnecessary. Gavage is a well accepted method for orally delivering agents found in drinking water. Dr. Dorman notes the gavage exposures are valuable for hazard characterization and relevant for that reason.	No changes needed.

	17) The SSAB states that there are “gaps” in the in vivo mutation studies (page 16, line 494). These studies offer the highest level of evidence for a non-mutagenic MOA because they are drinking water studies, they are performed at the carcinogenic dose, they assess mutation frequency in target tissue using validated endpoints, and they are GLP designs (Thompson et al., 2015a, 2017; Aoki et al., 2019). The only possible gap in these studies is that they do not capture large DNA deletions; however, target tissue micronucleus studies detect such large chromosomal mutations, and these studies were negative (O’Brien et al., 2013; Thompson et al., 2015b).	Context (opportunity to provide additional context). The limitations referenced in the summary are expanded upon earlier in the document.	No changes needed.
	18) The SSAB memorandum recommends using the NTP (2008) rodent bioassay data for risk assessment, but the tumors observed in the small intestine of the NTP study occurred only at high doses that caused prolonged cytotoxicity (Thompson et al., 2018). Specifically, female mice exposed to 5 and 20 ppm Cr(VI) continuously for 2 years did not exhibit statistically significant increases in intestinal tumors. Similarly, male mice did not exhibit statistically significant increases in tumors at drinking water exposures of 5 and 10 ppm Cr(VI). Thus, tumors were observed only in male mice at 30 ppm and in female mice at 60 ppm. Further, male and female rats exposed to Cr(VI) in drinking water at 180 ppm Cr(VI) did not develop intestinal tumors (NTP, 2008). In the MOA research study investigations (O’Brien et al., 2013; Thompson et al., 2015a,b) there was no evidence of genotoxicity or mutagenicity in the small intestine. EPRI recommends clarification for consistency in that there is recognition in the SSAB memorandum that the tumors observed in the target tissue of the NTP study were induced at doses that cause cytotoxicity (a threshold effect), but a subsequent recommendation that a linear model be used with these data because of the potential for low-dose mutagenicity.	Context (opportunity to provide additional context). All of the references cited in ths comment are discussed in detail in the SSAB recommendations, including the doses in each study.	No changes needed.

	19) These data are supplemented by OECD guideline-compliant in vivo transgenic mutation assays, which found no evidence of increased mutant frequency in the duodenum of mice or rats exposed to concentrations up to 180 ppm (Aoki et al., 2019; Thompson et al., 2017). Therefore, the available science does not support low-dose mutagenicity for either oral cavity or intestinal tumors. Further, there is no evidence of tumors at low inhalation exposure concentrations in either rodent studies or occupational epidemiology studies (Proctor et al., 2014). The SSAB postulated that there could be a duel MOA wherein Cr(VI) causes tumors by a mutagenic MOA at all doses, and tumors by a cytotoxic MOA only at high exposures; the scientific support for this theory requires clarification.	Perspective	No changes needed.
	20) ... it has been well recognized that low doses of Cr(VI) are reduced to the trivalent state by natural reducing agents in blood and extracellular fluid, such that reduction occurring prior to cellular absorption is a detoxifying process. .... This is a relevant consideration in the low-dose extrapolation methods used for risk assessment of Cr(VI), even if toxicokinetic models are not explicitly considered for risk assessment. The toxicokinetics of Cr(VI) provide a strong basis for non-linearity in the risk assessment model, as evaluated by the TCEQ (Haney et al., 2012, 2014; TCEQ, 2014, 2016) and Health Canada (2016) for both inhalation and oral exposures. It does not appear that the SSAB has considered this well-recognized biological process that is relevant to low-dose linearity. For example, on page 8 is the statement: *In the presence of uncertainty concerning target tissue concentrations of Cr(VI), it is health protective to assume that the entire amount reaching the target tissue/organ is in the more toxic Cr(VI) toxic form associated with the dichromate compound exposure. If incorrect, this will have the effect of overestimating dose to target tissue and hence risk*. EPRI recommends that this statement should be corrected, since if dose is overestimated, risk will be underestimated.	Re: the EPRI comment, *It does not appear that the SSAB has considered this well-recognized biological process that is relevant to low-dose linearity,* Dr. Kimble offers that the SSAB did consider this biological process. In the article by Kirman et al. (2013), referenced in the same paragraph of the draft SSAB document, the article discusses chromium reduction. Specifically, the article lists several uncertainties in the model including: "the rate of Cr(VI) reduction in human gastric contents estimated is based upon samples from fasted individuals", potential reducing agents may differ based on fasted vs fed, data lacking for human gastric samples with a pH of 4-7, data lacking for Cr(VI) reduction in the small intestine. Therefore, there are still uncertainties. Re: the EPRI comment, *For example, on page 8 is the statement: In the presence of uncertainty concerning target tissue concentrations of Cr(VI), it is health protective to assume that the entire amount reaching the target tissue/organ is in the more toxic Cr(VI) toxic form associated with the dichromate compound exposure. If incorrect, this will have the effect of overestimating dose to target tissue and hence risk. EPRI recommends that this statement should be corrected, since if dose is overestimated, risk will be underestimated,* Dr. Kimble relayed the original sentence doesn't need to be corrected. If the dose is overestimated, then the risk will be overestimated as well. Perhaps the sentenced could be modified to read something like "If incorrect, this will have the effect of overestimating dose to target tissue, which correspondingly leads to an overestimation of risk.". Drs. Starr and Dorman indicated it would depend on whether the application was to modeling or to risk characterization. Drs. Starr, Kenyon and Dorman suggested we delete the sentence at this early point in the document unless it's needed for sentences before/after.	Sentences deleted in proposed final version as they were not integral to the SSAB's recommendations in response the specific charge.

	21) The SSAB continues to discuss physiologically based pharmacokinetic (PBPK) models, stating on page 8: *Use of a PBPK mode for dose-response assessment in support of health-protective exposure limit development is most reliably accomplished through an independent review and evaluation of all aspects of the model, including: source and reliability of physiological and chemical-specific, assumptions regarding tissue transport…. (McLanahan et al. 2012*) EPRI notes that the use of PBPK models is favored in the EPA Cancer Risk Assessment Guidance (2005), which states that "physiologically based toxicokinetic modeling is potentially the most comprehensive way to account for biological processes that determine internal dose" (page 3-5). In addition, the EPA independently reviewed the toxicokinetic data for Cr(VI) and developed PBPK models for risk assessment (Schlosser and Sasso, 2014; Sasso and Schlosser, 2015).	Context. Dr. Kimble relays that on page 3-5 of Guidelines for Carcinogen Risk Assessment (EPA, 2005), the entire paragraph that contains the statement referenced in in EPRI comment reads as follows: "In the absence of chemical-specific data, physiologically based toxicokinetic modeling is potentially the most comprehensive way to account for biological processes that determine internal dose. Physiologically based models commonly describe blood flow between physiological compartments and simulate the relationship between applied dose and internal dose. Toxicokinetic models generally need data on absorption, distribution, metabolism, and elimination of the administered agent and its metabolites."	No changes needed.
Ari Lewis National Ash Management Advisory Board 05/20/2020	1)…, the SSAB made an overly cautious recommendation that is not supported by the best available science.	Perspective; no new science or critical evaluation of the draft analyses and recommendations presented for the differing perspective	No changes needed.
	2) Overall, the SSAB's decision to rely on a linear dose-response relationship to characterize Cr(VI) carcinogenicity is not scientifically justified and inconsistent with the evidence presented in its own evaluation.	Perspective; no new science or critical evaluation of the draft analyses and recommendations presented for the differing perspective	No changes needed.
	3) The most relevant studies for developing an oral carcinogenicity toxicity factor for Cr(VI) are drinking water studies that examine effects in target organs. While the SSAB presented the results from these critical studies, it did not prioritize this information when making its recommendations for a linear extrapolation approach. Instead, the SSAB relied heavily on genotoxicity results in non-target organs and from exposure routes that are not relevant to the human ingestion of drinking water.	One of the Board's summary statements notes data to prioritize among the many types of studies we reviewed... "2) Given currently available evidence, the State should base health protective goals on the highest quality lifetime studies in rodents (e.g., National Toxicology Program bioassays) and place the greatest emphasis on studies of rodent tumor responses and the mode of action by which these adverse effects developed. Particularly important are mechanistic studies in similar human tissues along with associated pharmacokinetics information to help with cross-species extrapolation." The tumors and mixed positive / negative micronucleus results which influenced our recommendations came from the NTP mammalian drinking water exposures we indicated to prioritize.	No changes needed.
	4) The studies that SSAB cites to support a mutagenic mode of action were all conducted before 2009.	Perspective. SSAB reviewed, considered, and cited references through 2019 and relied on all references in weighing evidence and making recommendations.	No changes needed.
	5) While the SSAB describes mode of action information published since 2010 as "substantial and robust" (Augspurger, 2020), most of the studies it mentions are only given a brief citation, and it is not clear that the SSAB has fully evaluated these studies.	Context. There is an opportunity to expand this section in the final to illustrate the depth of SSAB's consideration of a cytotoxic MOA (we received presentations and discussed them, we shared and discussed more than a dozen recent papers, considered them, and cited them). Many of the studies used to purport a non-primary mutagenic mode of actions are summarized earlier in the document (e.g., O'Brien et al. 2013; Thompson et al. 2015a; Aoki et al. 2019)	The *Cytotoxic mode of action* section was expanded. While some the new material is repetitive, we agree that it helps to reiterate it in this section.

	6) it is unclear why the SSAB has not given more weight to this more recent comprehensive analysis (Health Canada, 2016). In their consultation document leading up to the establishment of a revised drinking water guideline from Cr(VI), Health Canada described the confidence in the nonlinear MOA as "high"	Perspective. The Health Canada document did not review evidence for a mutagenic MOA. The SSAB draft references the Health Canada document, and the SSAB received an invited presentation on their work. The SSAB draft notes the documents we've weighed most heavily.	The introduction section of the SSAB's recommendations, which previously stated that SSAB received presentations on the topic, was expanded to list the entities which presented to the board during their Cr(VI) deliberations (North Carolina DEQ and DHHS, Texas Commission on Environmental Quality, New Jersey Department of Environmental Protection, California Environmental Protection Agency, ToxStrategies, and Health Canada).
	7) It is unclear what other information the SSAB would require to support a nonlinear extrapolation approach.	Context (opportunity to provide additional context)	Review of meeting minutes and discussions revealed no additional context was available on this point.
	8) The SSAB seems to provide some contradicting guidance. In Point 6 of its summary and conclusions, the SSAB notes that due to the uncertainties and because it could not "definitively chose among the modes of action" (Augspurger, 2020), the State should explore both linear and nonlinear extrapolation approaches (i.e., reference dose [RfD] and oral slope factor [OSF]) when developing a quantitative toxicity criterion from Cr(VI). Then, in the next sentence, it recommends only a linear approach. It notes that the selection of a linear extrapolation approach is a "science guided policy" (Augspurger, 2020). It is unclear what the basis for this "policy" decision.	Dr. Kimble relayed that she does not read this as contradictory since the statements indicate that the SSAB encourages the state to explore both, while the majority view of the SSAB is that the state consider a linear approach. Perhaps a slight re-wording like “Due to the remaining uncertainty and because it is generally considered to be a more health-protective approach, the SSAB recommends the state consider a linear extrapolation approach (this was a majority view; one member thought no recommendations should be offered).” We could clarify the recommendation to follow dual routes in the body of the review and our recommendations section which reiterates this but advances one path (per our charge).	Rephrased in proposed final as suggested during review.
	9) Because US EPA will have the time and resources to fully contemplate the wealth of mode-of-action information that has been developed since its last review of Cr(VI) carcinogenicity in 2010, it would be advisable for any state agency to wait for US EPA to make a determination on a linear vs nonlinear extrapolation approach for Cr(VI).	Perspective. The utility of EPA's on-going systematic review is mentioned at the beginning and end of the SSAB's recommendation document. We will check the proposed date of EPA's proposed FY21 public review draft and update the link if needed.	EPA's proposed time for public review draft availability is updated in the proposed final recommendations (now 4th quarter FY21).
	10) The state of the science clearly gives weight to a non-mutagenic mode of action for Cr(VI) in relevant target organs, which supports a nonlinear extrapolation approach.	Perspective	No changes needed.
	11) At a minimum, both a linear and a nonlinear approach should be explored when developing quantitative toxicity criteria for Cr(VI), although more weight should be given the more scientifically supportable nonlinear approach.	Perspective. The SSAB indicated the value of exploring both approaches in their recommendations section.	No changes needed.
Zach Hall, Director – Environmental Science Duke Energy 5/29/2020 (references the EPRI and NAMAB comments)	1) Duke Energy does not believe that the SSABs’ decision to rely on a linear dose relationship to characterize hexavalent chromium carcinogenicity is scientifically justified.	Perspective; no new science or critical evaluation of the draft analyses and recommendations presented for the differing perspective	No changes needed.
	2) …. the evidence presented in SSAB’s memo does not support the use of a linear dose relationship.	Perspective; no new science or critical evaluation of the draft analyses and recommendations presented for the differing perspective	No changes needed.

	3) The current state of the science specifically points towards a non-linear extrapolation approach as the most well supported methodology.	Perspective; no new science or critical evaluation of the draft analyses and recommendations presented for the differing perspective	No changes needed.
Hope C. Taylor, Executive Director Clean Water for North Carolina 6/1/2020	1) Despite the limited drinking water studies to indicate a mutagenic mechanism of action, the overwhelming evidence of mutagenicity via inhalation exposure in humans means we simply can't rule out mutagenicity and must, therefore, apply a dose response model that mandates the more precautionary approach to human exposures.	Perspective	No changes needed.