Memorandum

Date: DRAFT

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 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'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 with mutagenic action in inhalation exposures (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

[1] A mutation is a heritable change in the DNA sequence, a common early event in tumor development. Genotoxicity is damage to the genetic material by a chemical agent. All mutagens are genotoxic, but not all genotoxins are mutagenic (heritable).

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).

"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

[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.

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®

[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.

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 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. 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

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

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 $C57BL/6J p^{un}/p^{un}$ 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 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. 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 mutations (Group A carcinogen). 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. 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 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. 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 IRIS timeline (https://www.epa.gov/iris/iris-program-outlook), the target date for the Cr(VI) Public Comment Draft is spring 2021.

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