

1 **Memorandum**

2
3 **Date:** DRAFT

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

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

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

13
14 **Subject:** Secretaries' Science Advisory Board response to inquiry on hexavalent chromium
15

16 **Background**

17
18 Two duties of the [Secretaries' Science Advisory Board](#) (SSAB) are to act as consultants to the
19 North Carolina Department of Environmental Quality (DEQ) on factors for establishing
20 acceptable levels of contaminants and to provide input to the North Carolina Department of
21 Health and Human Services (DHHS) as they establish health goals. In June 2018, DEQ and
22 DHHS requested the SSAB's review and recommendations on hexavalent chromium [Cr(VI)]
23 science to use for developing public health and environmental standards. In December 2018,
24 the charge to the SSAB was refined as follows:

25
26 *DEQ and DHHS requests the SSAB review the current hexavalent chromium*
27 *toxicological science related to a linear versus a non-linear exposure response and*
28 *provide recommendations to the appropriate science to be used for development of*
29 *regulatory standards protective of public health and the environment for groundwater*
30 *and surface water.*

31
32 This memorandum conveys the SSAB's response to that specific charge. A decision to select
33 a linear or a non-linear dose-response model for oral exposures to Cr(VI) is informed by
34 consideration of the toxicological and epidemiological evidence, particularly as it informs
35 mode(s) of action. A mutagenic mode of action in carcinogenesis would typically lead to
36 assumption of a linear no-threshold approach to dose-response assessment (resulting in
37 calculation of an oral slope factor, OSF) whereas a non-mutagenic (e.g., effects due to

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

48

49 **Approach and Analysis**

50 The SSAB received scientific data and information from Federal, State, and international
51 government agencies, from a consulting company to industry stakeholders, and by members
52 of the public. The materials presented and a summary of the discussions during the
53 presentations are found on the SSAB website ([https://deq.nc.gov/about/boards-and-](https://deq.nc.gov/about/boards-and-commissions/secretaries-science-advisory-board)
54 [commissions/secretaries-science-advisory-board](https://deq.nc.gov/about/boards-and-commissions/secretaries-science-advisory-board)). The reader is directed to that publicly
55 available website for specific information as well as audio files of the presentations and
56 discussions. The information provided to the SSAB was useful but note that a critical review
57 of the presentations has not occurred, nor has the SSAB conducted a detailed quality
58 evaluation of all the scientific studies summarized below.

59

60 The SSAB's review focused on research, reviews and syntheses conducted over the last fifteen
61 years, a period of active investigation on the mode or modes of action of Cr(VI) toxicity
62 following National Toxicology Program (NTP 2007 and 2008) drinking water studies in mice
63 and rats which reported tumors evidencing carcinogenic activity and other effects. The SSAB
64 reviewed independently and discussed current literature and recent syntheses related to hazard
65 assessment of Cr(VI) in drinking water. We note the value of recent syntheses (e.g.,
66 McCarroll et al. 2010; Stern 2010; USEPA 2010; ATSDR 2012; Zhitkovich 2011; Haney
67 2015a-c; Sun et al. 2015; Health Canada 2016; Thompson et al. 2013, 2014, 2017a, 2018; Suh
68 et al. 2019) which examine and evaluate the weight of evidence for linear and non-linear

69 modeling approaches to existing data as the most relevant to the charge from DEQ and
70 DHHS. There are also highly relevant mode of action studies (e.g., O'Brien et al. 2013;
71 Thompson et al. 2015a-c, 2017b; Aoki et al. 2019), many but not all of which are referenced
72 in the hazard assessment syntheses. With over 1,000 potentially relevant papers on Cr(VI)
73 mode of action, each new synthesis has the opportunity to build on recent data. We note an
74 on-going systematic review of the mutagenic potential of orally administered Cr(VI) (USEPA
75 2019) as an opportunity to have refinement of the following analysis and recommendations
76 when the USEPA analysis is completed.

77

78 We derived recommendations following the USEPA's Guidelines for Carcinogen Risk
79 Assessment (USEPA 2005) and Guidelines for Mutagenicity Risk Assessment (USEPA
80 1986). The 2005 USEPA guidelines state:

81 "When the weight of evidence evaluation of all available data are insufficient to
82 establish the mode of action for a tumor site and when scientifically plausible based on
83 the available data, linear extrapolation is used as a default approach, because linear
84 extrapolation generally is considered to be a health-protective approach. Nonlinear
85 approaches generally should not be used in cases where the mode of action has not
86 been ascertained. Where alternative approaches with significant biological support are
87 available for the same tumor response and no scientific consensus favors a single
88 approach, an assessment may present results based on more than one approach.

89

90 A *nonlinear* approach should be selected when there are sufficient data to ascertain
91 mode of action and conclude that it is not linear at low doses and the agent does not
92 demonstrate mutagenic or other activity consistent with linearity at low doses. Special
93 attention is important when the data support a nonlinear mode of action but there is
94 also a suggestion of mutagenicity. Depending on the strength of the suggestion of
95 mutagenicity, the assessment may justify a conclusion that mutagenicity is not
96 operative at low doses and focus on a nonlinear approach, or alternatively, the
97 assessment may use both linear and nonlinear approaches.

98

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

109

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

113

114 *Cancer and other endpoints in key primary references*

115 Evidence regarding Cr(VI) carcinogenesis comes from both human epidemiological and
116 animal studies. For example, Cr(VI) is a recognized human carcinogen with mutagenic action
117 in inhalation exposures (IARC 2012). The NTP has classified Cr(VI) as a known human
118 carcinogen based on sufficient evidence of carcinogenicity from studies in humans (NTP
119 Report on Carcinogens, Fourteenth Edition see:

120 <https://ntp.niehs.nih.gov/ntp/roc/content/profiles/chromiumhexavalentcompounds.pdf>). This
121 determination is largely based on occupational cohorts exposed to Cr(VI) via inhalation.

122

123 A two-year NTP (2008) bioassay exposed male and female rats and mice to dichromate
124 dihydrate in drinking water. Rats were exposed to drinking water containing 0, 14.3, 57.3,
125 172, or 516 mg/L sodium dichromate dihydrate (equivalent to 0, 5, 20, 60, or 180 mg/L
126 hexavalent chromium) for 2 years (equivalent to average daily doses of approximately 0.6,
127 2.2, 6, or 17 mg sodium dichromate dihydrate/kg body weight for males and 0.7, 2.7, 7, or 20
128 mg/kg for females). Male mice were exposed to drinking water containing 0, 14.3, 28.6, 85.7,
129 or 257.4 mg/L sodium dichromate dihydrate (equivalent to 0, 5, 10, 30, or 90 mg/L hexavalent
130 chromium) for 2 years (equivalent to average daily doses of approximately 1.1, 2.6, 7, or 17
131 mg sodium dichromate dihydrate/kg body weight). Female mice were exposed to drinking
132 water containing 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate (equivalent to
133 0, 5, 20, 60, or 180 mg/L hexavalent chromium) for 2 years (equivalent to average daily doses
134 of approximately 1.1, 3.9, 9, or 25 mg/kg hexavalent chromium).

135

136 Exposure of rodents to Cr(VI) was associated with decreased body weight and water
137 consumption that was secondary to palatability issues. Mean body weights of 516 mg/L
138 sodium dichromate dihydrate (180 mg/L hexavalent chromium) males and female rats were
139 less than those of the controls throughout the study. Water consumption by 172 and 516 mg/L
140 sodium dichromate dihydrate rats was less than that by the controls throughout the study.

141 Terminal mean body weight of 172 mg/L sodium dichromate dihydrate (60 mg/L hexavalent
142 chromium) female mice was 8% less than that of the controls, and the mean body weight of
143 516 mg/L female mice was 15% less than that of the controls. Water consumption by 85.7
144 and 257.4 mg/L sodium dichromate dihydrate males and 172 and 516 mg/L sodium
145 dichromate dihydrate female mice was less than that by the controls throughout the study.

146

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

154

155 Neoplasms of the small intestine (duodenum, jejunum, or ileum) were seen in exposed male
156 and female mice. The incidences of adenoma of the duodenum in 257.4 mg/L males and 172
157 and 516 mg/L female mice were significantly greater than those in the controls. The
158 incidence of carcinoma of the duodenum was statistically significantly increased in 516 mg/L
159 female mice. The incidence of adenoma of the jejunum in 516 mg/L female mice was
160 significantly increased compared to that in the controls. When the incidences of adenoma and
161 carcinoma tumors were combined for all sites of the small intestine, the incidences were
162 statistically significantly increased in 85.7 and 257.4 mg/L males and 172 and 516 mg/L
163 females compared to those in the controls. The incidences in 57.3 mg/L females exceeded the
164 historical control ranges for drinking water studies and for all routes of administration. The
165 incidences of diffuse epithelial hyperplasia were significantly increased in the duodenum of
166 all exposed groups of male and female mice. The incidences of histiocytic cellular infiltration
167 were significantly increased in the duodenum of 85.7 and 257.4 mg/L males and in 172 and
168 516 mg/L females. In the jejunum, the incidences of diffuse epithelial hyperplasia and
169 histiocytic cellular infiltration were significantly increased in 516 mg/L females. The
170 incidences of histiocytic cellular infiltration of the liver in all exposed groups of females, of
171 the mesenteric lymph node in all exposed groups of males and females, and of the pancreatic

172 lymph node of 85.7 and 257.4 mg/L males and 172 and 516 mg/L females were significantly
173 increased.

174

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

181

182 The NTP (2008) concluded that there was clear evidence of carcinogenic activity of sodium
183 dichromate dihydrate exposure via drinking water in male and female F344/N rats based on
184 increased incidences of squamous cell neoplasms of the oral cavity. There was clear evidence
185 of carcinogenic activity of Cr(VI) associated with the sodium dichromate dihydrate exposure
186 in male and female B6C3F1 mice based on increased incidences of neoplasms of the small
187 intestine (duodenum, jejunum, or ileum). Exposure to sodium dichromate dihydrate also
188 resulted in histiocytic cellular infiltration in the liver, small intestine, and pancreatic and
189 mesenteric lymph nodes of rats and mice and diffuse epithelial hyperplasia in the small
190 intestine of male and female mice.

191

192 *Dose-response modeling*

193 This section focuses on issues pertinent to disposition of chromium in the body and dose-
194 response for the oral route of exposure. Chromium, like many other metals, undergoes
195 valence state shifts rather than enzymatically catalyzed biotransformation. Trivalent
196 chromium [Cr(III)] is an essential element associated with carbohydrate metabolism, whereas
197 Cr(VI) is classified as a known human carcinogen in the lung. Gastric juices reduce Cr(VI) to
198 Cr(III) via a 2nd-order reaction *in vitro*. Total reducing capacity in all mammalian species is
199 generally between 10–30 mg/L gastric contents. Components of gastric juice reducing Cr(VI)
200 include ascorbate, glutathione, NADH, and sulfhydryls. Reduction rate decreases as pH
201 increases (De Flora et al. 1997; Proctor et al. 2012; Kirman et al. 2013). This is an important
202 consideration due to differences in stomach structure and pH between rodents and humans.

203 Transport of Cr(VI) occurs rapidly by unspecified phosphate and sulfate active transporters
204 (Alexander and Aaseth 1995) whereas transport of Cr(III) occurs more slowly via diffusion.
205 Gastrointestinal absorption rates are highly variable for both Cr(VI) and Cr(III). Uptake of
206 Cr(VI) from the gut lumen is rapid and systemic reduction to Cr(III) is also rapid. Once
207 reduced, Cr(III) will diffuse slowly into or out of tissues, and distribute to tissues in plasma.

208

209 Both the uptake and reduction of Cr(VI) by red blood cells (RBCs) are estimated to be rapid
210 (Devoy et al. 2016). Because Cr(III) exhibits a lower rate of transport through cellular
211 membranes than Cr(VI), Cr(III) remains trapped in RBCs. The RBC to plasma ratio has been
212 used to indirectly infer cellular uptake and partitioning (and hence distribution and
213 absorption), although this becomes unreliable if ratios exceed 1 as may occur following high
214 acute or chronic exposure (Kirman et al. 2013). Only total chromium can be reliably
215 measured in tissues. In evaluating dose-response relationships for chromium, uncertainty
216 related to tissue speciation needs to be explicitly considered.

217

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

227

228 The ability to evaluate the relationship between external exposure and internal dose is
229 uncertain for Cr because analytical technology available to speciate the metal is limiting. In
230 the case of chromium, only total chromium (the sum of all present valence states) can be
231 reliably measured in tissues, where as Cr(VI) and Cr(III) can be reliably speciated in aqueous
232 systems. Cr(VI) membrane transport is carrier-mediated, whereas Cr(III) transport is via
233 diffusion. Based on differences in cellular uptake and partitioning, speciation (and hence

234 distribution and absorption) can be indirectly inferred based on red blood cell to plasma ratio,
235 although this becomes unreliable if ratios exceed 1 (Kirman et al. 2013). In evaluating dose-
236 response relationships for chromium, uncertainty related to speciation needs to be explicitly
237 considered limited. In the presence of uncertainty concerning target tissue concentration of
238 Cr(VI), it is health protective to assume that the entire amount reaching the target tissue/organ
239 is in the more toxic Cr(VI) toxic form associated with the dichromate compound exposures.
240 If incorrect, this will have the effect of overestimating dose to target tissue and hence risk.
241 This would be the operative assumption if dose-response analysis is conducted using
242 administered dose (e.g. concentration in drinking water) rather than dose of Cr(VI) reaching
243 the target tissue.

244

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

256

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

263

264

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

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

270

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

273 “Determination of carcinogens that are operating by a mutagenic mode of action, for
274 example, entails evaluation of in vivo or in vitro short-term testing results for genetic
275 endpoints, metabolic profiles, physicochemical properties, and structure-activity
276 relationship (SAR) analyses in a weight-of-evidence approach (Dearfield et al. 1991;
277 U.S. EPA, 1986b; Waters et al. 1999). Key data for a mutagenic mode of action may
278 be evidence that the carcinogen or a metabolite is DNA-reactive and/or has the ability
279 to bind to DNA. Also, mutagenic carcinogens usually produce positive effects in
280 multiple test systems for different genetic endpoints, particularly gene mutations and
281 structural chromosome aberrations, and in tests performed in vivo which generally are
282 supported by positive tests in vitro.” USEPA Guidelines pp 2-30.

283

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

287

288 *Evaluation of evidence*

289 Evidence for the mutagenicity¹ of Cr(VI) is extensive and complex. The evidence to be
290 considered includes the following:

291

292 Mutagenic endpoints “include point mutations (i.e., submicroscopic changes in the base
293 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).

294 aberrations include deficiencies, duplications, insertions, inversions, and translocations,
295 whereas numerical aberrations are gains or losses of whole chromosomes (e.g., trisomy,
296 monosomy) or sets of chromosomes (haploidy, polyploidy). Certain mutagens, such as
297 alkylating agents, can directly induce alterations in the DNA. Mutagenic effects may
298 also come about through mechanisms other than chemical alterations of DNA
299 (“epigenetic² modifications”). Among these are interference with normal DNA
300 synthesis (as caused by some metal mutagens), interference with DNA repair, abnormal
301 DNA methylation, abnormal nuclear division processes, or lesions in non-DNA targets
302 (e.g., protamine, tubulin).” (USEPA Guidelines for Mutagenicity Risk Assessment pp
303 4).

304
305 “In evaluating chemicals for mutagenic activity, a number of factors will be considered:
306 (1) genetic endpoints (e.g., gene mutations, structural or numerical chromosomal
307 aberrations) detected by the test systems, (2) sensitivity and predictive value of the test
308 systems for various classes of chemical compounds, (3) number of different test
309 systems used for detecting each genetic endpoint, (4) consistency of the results obtained
310 in different test systems and different species, (5) aspects of the dose-response
311 relationship, and (6) whether the tests are conducted in accordance with appropriate test
312 protocols agreed upon by experts in the field.” USEPA Guidelines for Mutagenicity
313 Risk Assessment pp 8).

314

315 Results from laboratory animal studies are judged to be informative as indicated by USEPA
316 (1986):

317 Despite species differences in metabolism, DNA repair, and other physiological
318 processes affecting chemical mutagenesis, the virtual universality of DNA as the
319 genetic material and of the genetic code provides a rationale for using various
320 nonhuman test systems to predict the intrinsic mutagenicity of test chemicals.

321 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.

322 that chemicals causing genetic effects in one species or test system frequently cause
323 similar effects in other species or systems.

324

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

337

338 Oral exposures via drinking water

339 Three studies (O'Brien et al. 2013; Thompson et al. 2015a; Aoki et al. 2019) have been
340 published that specifically looked for increased mutation frequency in tumor target tissues in
341 rodents. Sodium dichromate dehydrate exposed B6C3F₁ mice (0.3–520 mg/L in drinking
342 water for 7 and 90 days) showed no increased K-Ras³ codon 12 GAT mutations in duodenum
343 (O'Brien et al. 2013). Exposure of Big Blue® TgF344 rats to 180 mg/L Cr(VI) in drinking
344 water for 28 days did not significantly increase the mutant frequency in the *cII* transgene in
345 the gingival/buccal or the gingival/palate regions relative to controls (Thompson et al. 2015a).
346 Sodium dichromate dihydrate was administered orally in drinking water to male *gpt* delta
347 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
348 days; no significant increase in *gpt* mutant frequency relative to that in control mice was
349 observed in the small intestine (Aoki et al. 2019). Two of the studies (Thompson et al. 2015a
350 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.

351 rats and *gpt* delta transgenic mice); these systems can detect point mutations and small-scale
352 deletions but are not sensitive to larger deletions or aneuploidy (gain or loss of whole
353 chromosomes). The O'Brien et al. (2013) study (in mice) only looked for mutations at *K-Ras*
354 codon 12. Codon 12 is one of several codons in *K-Ras* that have been implicated in human
355 colon cancers, and *K-Ras* is one of several oncogenes⁴ known to be mutated in human colon
356 cancer.

357

358 The results of micronuclei from rodent drinking water studies are mixed positive and negative
359 (Mirsalis et al. 1996; De Flora et al. 2006; NTP 2007; O'Brien et al. 2013; Thompson et al.
360 2015b). Mirsalis et al. (1996) reported no statistically significant increase in micronucleated
361 RNA-positive erythrocytes in mice allowed ad libitum access to drinking water with up to 20
362 mg/L Cr(VI) for 48 hr. De Flora et al. (2006) reported no increase of the micronucleus
363 frequency in bone marrow or peripheral blood erythrocytes of mice exposed to sodium
364 dichromate dihydrate and potassium dichromate administered with drinking water up to a
365 concentration of 500 mg/L Cr(VI) for up to 210 days. NTP (2007) summarize two studies. In
366 study 1, male and female B6C3F₁ mice were given drinking water containing up to 1,000 mg
367 sodium dichromate dihydrate/L for 3 months. No significant increases were seen in
368 micronucleated normochromatic erythrocytes in peripheral blood samples. In study 2,
369 micronucleus frequencies were evaluated in male B6C3F₁, BALB/c, and *am3-C57BL/6* mice
370 administered sodium dichromate dihydrate up to 250 mg/L in drinking water for 3 months. A
371 significant exposure concentration-related increase in micronucleated normochromatic
372 erythrocytes was seen in *am3-C57BL/6* male mice (in two of the three exposed groups of this
373 strain, micronuclei were significantly elevated). An increase in micronucleated erythrocytes
374 was noted in male B6C3F₁ mice but judged by the authors to be "equivocal" based on a small
375 increase in micronuclei of exposed groups that did not reach statistical significance above the
376 control group. No increase in micronucleated normochromatic erythrocytes was observed in
377 male BALB/c mice (NTP 2007). No exposure-related effects on the percentage of
378 polychromatic erythrocytes was observed in any of the three mouse strains tested. Concerns
379 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.

380 in *am3-c57BL/6* male mice with results judged "equivocal" in the B6C3F₁ mouse strain that
381 has typically been used for NTP carcinogenicity testing.

382

383 O'Brien et al. (2013) report that sodium dichromate dehydrate exposed B6C3F₁ mice (0.3–
384 520 mg/L in drinking water for 7 and 90 days) showed no increased micronuclei and
385 karyorrhectic nuclei in the duodenal crypts. Thompson et al. (2015b) report Cr(VI), in the
386 form of sodium dichromate dehydrate in drinking water up to 180 ppm for 7 days, did not
387 increase micronuclei in female B6C3F₁ mice.

388

389 Other endpoints from Cr(VI) exposures via drinking water include DNA deletions which were
390 positive (Kirpnick-Sobol et al. 2006). Pregnant C57BL/6J p^{un}/p^{un} mice were given free
391 access to Cr-supplemented drinking water (potassium dichromate used at 62.5 or 125.0 mg/L,
392 and 20-day-old offspring were harvested to examine for DNA deletions. In this model, a
393 somatic deletion reconstitutes the wild-type p gene, resulting in black-pigmented cells
394 (eyespot) on the retinal pigment epithelium. Offspring of mice treated with Cr(VI) had
395 statistically-significant increases in the number of eyespots on the retinal epithelium, that
396 study's measure of the frequency of DNA deletions. The background (control) eyespot
397 frequency was significantly increased by 27% and 38% in the treated groups, respectively,
398 although the treated group frequencies were not significantly different from one another.
399 Concerns include that exposures of embryos was transplacental during a highly sensitive 10
400 day period in their development (the mother received Cr(VI) via drinking water, but the assay
401 was of the offspring). Also, there was no significant dose-response in the treated groups,
402 sample sizes of the treated groups were markedly lower (n=24 and 14) versus the n=55 for the
403 control group (this discrepancy in sample sizes is not explained and could be a source of bias),
404 and a scan of PubMed failed to reveal other studies that have replicated this finding.

405

406 In other Cr(VI) drinking water studies, DNA double-strand breaks are negative (Thompson et.
407 al. 2015c; Sánchez-Martín et al. 2015); DNA protein cross-links are negative (De Flora et al.
408 2008; Coogan et al. 1991); increased complexing of proteins with DNA was demonstrated in
409 liver following 3 weeks of exposure at both 100 and 200 ppm chromium (Coogan et al. 1991),
410 and unscheduled DNA synthesis was negative (Mirsalis et al. 1996).

411

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

416

417 Oral exposures via gavage

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

423

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

427

428 Intratracheal and inhalation exposures

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

431

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

435

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

440

441 Intraperitoneal exposures

442 At least 14 studies by multiple investigators have been published, all of which indicated
443 positive results for mutation frequency, dominant lethal mutations, micronuclei, DNA damage
444 via the comet assay, or suppressed nuclear DNA synthesis (Wild 1978; Knudsen 1980;
445 Amlacher and Rudolph 1981; Hayashi et al. 1982; Paschin and Toropzev 1982; Paschin et al.
446 1982; Shindo et al. 1989; Itoh and Shimada 1996, 1997, 1998; Wronska-Nofer et al. 1999;
447 Sekihashi et al. 2001; Ueno et al. 2001; De Flora et al. 2006).

448

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

453

454 *Studies of specimens collected from humans*

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

462

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

464 In certain circumstances, the 2005 USEPA Guidelines for Carcinogen Risk Assessment allow
465 for a non-linear dose-response assessment as a plausible alternative to the default “linear
466 through zero” assessment utilizing a linearized multi-stage model analysis of tumor incidence
467 data. These circumstances include 1) significant evidence of a tumor response at only one or
468 two of the highest doses in a cancer bioassay, with little or no evidence of a tumor response at
469 the lower doses; 2) significant evidence of related cytotoxicity and enhanced restorative cell
470 proliferation in the target tissues at the same highest doses and temporally preceding the
471 tumor responses, again with little or no evidence of this precursor response at the lower doses;
472 and 3) little or no evidence of *in vivo* genotoxicity in the target tissues. Physiologically-based

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

477

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

487

488 *Comparative weight of evidence for potentially relevant modes of action*

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

498

499 The data from human studies clearly show that Cr(VI) via inhalation can cause mutations
500 (Group A carcinogen). There is a paucity of studies from human exposures to Cr(VI) via
501 drinking water.

502

503 The case can be made for a non-linear dose-response assessment for Cr(VI) carcinogenicity as
504 a plausible alternative to EPA’s default “linear through zero” approach to the assessment of
505 genotoxic carcinogens. Recent references for a cytotoxic mode of action identified using
506 PubMed include Kopec et al. 2011; Proctor et al. 2011, 2012; Thompson et al. 2011a, b,
507 2012a-c, 2013, 2014, 2015a-c, 2016a, b, 2017a-c, 2018; O’Brien et al. 2013; Suh et al. 2014,
508 2019; Rager et al. 2017; and Aoki et al. 2019. The database is substantial and robust. It
509 includes more than two dozen peer-reviewed publications that describe how a non-linear
510 assessment was developed by acquiring extensive mechanistic data relevant to Cr(VI)
511 carcinogenicity. A non-linear dose-response assessment merits serious consideration.
512 Mutagenicity data for Cr(VI) in the oral mucosa and duodenum of Big Blue® rats exposed to
513 Cr(VI) in drinking water are negative (Thompson et al. 2015a, 2017b). Furthermore, there
514 were no dose-related increases in *K-Ras* mutant frequency, micronuclei formation, or change
515 in mitotic or apoptotic indices in crypt tissues taken from mice exposed to Cr(VI) in drinking
516 water (O’Brien et al. 2013) and no significant increase in *gpt* mutant frequency in small
517 intestines of male *gpt* delta mice exposed to Cr(VI) in drinking water (Aoki et al. 2019). Gaps
518 in knowledge affect the confidence in conclusions that can be drawn about a mutagenic
519 (linear) mode of action and the potential for carcinogenesis from oral exposure to Cr(VI).

520

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

527 “Generally, “sufficient” support [regarding a carcinogenic mode of action] is a matter of
528 scientific judgment in the context of the requirements of the decision maker or in the
529 context of science policy guidance regarding a certain mode of action.” USEPA
530 Guidelines pp 2-42

531

532 **Summary and Recommendations**

533

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

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

556 3) The data from human studies clearly show that Cr(VI) exposure via inhalation can
557 cause mutations and cancer. In 2-year lifetime rodent studies, NTP concluded that there was
558 clear evidence of carcinogenic activity of Cr(VI) exposure via drinking water based on
559 observations of increased incidences of oral cavity tumors in male and female rats, and small
560 intestinal tumors in male and female mice. The evidence regarding the potential for a
561 mutagenic mode of action for Cr(VI) oral exposures is complex and difficult to interpret with
562 positive and negative findings and interpretation challenges due to the assays employed. The
563 available drinking water mutation frequency studies are negative. The results from drinking
564 water studies of micronuclei are mixed positive and negative; DNA deletions are positive;

565 DNA double-strand breaks are negative; DNA protein cross-links are mixed; and unscheduled
566 DNA synthesis are negative. Similarly, the rodent gavage studies are mixed with negative
567 results in micronuclei and positive findings studies of DNA damage using the comet assay.
568 The available intratracheal and intraperitoneal studies indicate Cr(VI) may cause mutations,
569 but there is uncertainty about absorption, distribution, metabolism and excretion of Cr(VI) via
570 these routes and hence interpretation of results.

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

587 5) The mixed positive and negative genotoxicity results from laboratory studies via
588 non-inhalation exposure routes, coupled with clear evidence in humans that Cr(VI) exposure
589 via inhalation is mutagenic and carcinogenic, provide evidence that a mutagenic mode of
590 action is potentially operative for Cr(VI) exposures via drinking water. However there is only
591 very limited evidence from Cr(VI) drinking water studies of a mutagenic mode of action.

592 6) Multiple modes of action may be occurring simultaneously and the sequence of
593 events leading to cancer formation is uncertain. Significant data gaps and uncertainties
594 remain (e.g., mode of action assessment in the few rodent drinking water studies address a
595 limited suite of endpoints, and there is evidence of mutagenic responses in tissues other than

596 where tumors occur). There is not conclusive evidence to rule out a mutagenic mode of
597 action, and we conclude that Cr(VI) via drinking water exposure may cause mutational
598 changes. Further, remaining uncertainties (e.g., physiologically-based pharmacokinetic
599 modeling) are such that we could not definitively choose among the modes of action, and
600 therefore quantitative dose response assessment leading to both an OSF and RfD should be
601 explored by the State. As a science guided policy, the SSAB recommends the State consider a
602 linear extrapolation approach because of the remaining uncertainty and because it generally is
603 considered to be a more health-protective approach (this was a majority view; one member
604 thought no science-guided policy recommendations should be offered).

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

611

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