Development of a Carcinogenic-Based RfD for Hexavalent Chromium (CrVI)

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Texas Commission on Environmental Quality
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This presentation represents my own views and opinions and not necessarily those of the TCEQ.

TCEQ Toxicology Division

- 15 busy, hard-working toxicologists/risk assessors
- Support most programs at the TCEQ
- For example, involved in:
  - ✓ Review of air data from the most extensive ambient air monitoring network in the nation, ≈ 95 air toxics sites (e.g., VOCs, PAHs, metals, carbonyls, H_2S).
  - ✓ Air permitting (TCAA requires all sources and emissions be authorized, even BBQ pits and water heaters).
  - ✓ Remediation risk assessment.
  - ✓ Risk communication (legislature, public, management, media).
  - ✓ Objective data analysis for policymakers.
  - ✓ *Toxicity factor development (e.g., CrVI URF, RfD).*

TCEQ Toxicity Factor Guidelines

- Guidelines were originally drafted in 2005
- External expert peer reviewed
- 2 rounds of public comment
- Finalized in 2006
- Updated version was drafted in 2011
- Also subjected to external expert peer review and public comment
- Finalized October 2012
- Both times the external review was organized by Toxicology Excellence for Risk Assessment (*TERA*) with diverse external experts from government (e.g., USEPA, CalEPA), academia (e.g., UC, NYUSM, UTSPH), consulting (e.g., David Gaylor, Bruce Allen, John Christopher), and others (e.g., Lovelace Respiratory Research Institute, NUATRC).
- Updated again in 2015 (323 page guidance document).
- *Our Goal: a state-of-the-science guidance document*.

Some Peer Reviewer Comments

"To the best of my knowledge, this guidance is complete and thorough, even exhaustive, in its coverage of relevant guidance on development of toxicity criteria available in the United States and Europe."

"This draft guidance is not just comprehensive, it is encyclopedic."

"The authors of this report are to be commended for the thoroughness, accuracy and usefulness instilled into this report."

Sound Science

- Our goal: Use state-of-the-science guidelines to derive scientifically-sound toxicity factors.

- Derivations can be found in Development Support Documents (DSDs) available on the web (https://www.tceq.texas.gov/toxicology/dsd/final.html).

- TCEQ has also published various derived values in the peer-reviewed scientific literature (e.g., 1,3-butadiene, nickel, arsenic, cadmium, CrVI, diethanolamine).

Sound Science

A bibliography of some papers by TCEQ toxicologists that have appeared in scientific journals:

1. Nancy B. Beck, Richard A. Becker, Neeraja Erraguntla, William H. Farland, Roberta L. Grant, George Gray, Christopher Kirman, Judy S. LaKind, R. Jeffrey Lewis, Patricia Nance, Lynn H. Pottenger, Susan L. Santos, Stephanie Shirley, Ted Simon, Michael L. Dourson. 2016. Approaches for describing and communicating overall uncertainty in toxicity characterizations: U.S. Environmental Protection Agency's Integrated Risk Information System (IRIS) as a case study. Environment International 89–90: 110–128.

2. Capobianco, T., S.M. Hildebrand, M. Honeycutt, J.S. Lee, D. McCant, and R.L. Grant. 2013. Impact of three interactive Texas state regulatory programs to decrease ambient air toxic Levels. J Air Waste Management Association 63(5): 507–20.

Open access: http://www.tandfonline.com/eprint/8SbFyWjzj4SPbje53BxP/full

3. Erraguntla, N.K. and R.L. Grant. 2015. Health- and vegetative-based effect screening values for ethylene. Chemico-Biological Interactions. Available online 26 February 2015.

4. Erraguntla, N.K., R.L. Sielken, C. Valdez-Flores, and R.L. Grant. 2012. An updated inhalation unit risk factor for arsenic and inorganic arsenic compounds based on a combined analysis of epidemiology studies. Regulatory Toxicology and Pharmacology 64: 329–41.

5. Ethridge, S., Bredfeldt, T., Sheedy, K., Shirley, S., Lopez, G., and M. Honeycutt. 2015. The Barnett Shale: From problem formulation to risk management. Journal of Unconventional Oil and Gas Resources 11: 95-110.

6. Goldstein, B.D., B.W. Brooks, S.D. Cohen, A.E. Gates, M.E. Honeycutt, J.B. Morris, T.M. Penning, J. Orme-Zavalets, and J. Snawder. 2014. The role of toxicological sciences in meeting the challenges and opportunities of hydraulic fracturing. Toxicological Sciences 139(2): 271–83

7. Goodman J.E., Sax S.N., Lange S., and L.R. Rhomberg. 2015. Are the elements of the proposed ozone National Ambient Air Quality Standards informed by the best available science? Regulatory Toxicology and Pharmacology 72(1): 134–40.

8. Grant, R.L., D. Morrill, D. Manis, A. Tachovsky, and L.C. Haws. 2000. The Texas combustion project: Review of trial burn/risk burn plans and reports. Proceedings of the Incineration and Thermal Treatment Technologies Conference (IT3 Conference).

9. Grant, R.L., R.J. Rodriguez, C.S. Hofelt and L.C. Haws. 2002. Shortcomings in USEPA approach for predicting risk due to consumption of animal food products impacted by air emissions from hazardous waste combustion facilities: A case study involving phthalates, Human & Ecological Risk Assess. 8: 1137–54.

10. Grant, R.L., V. Leopold, D. McCant, and M. Honeycutt. 2007. Spatial and temporal trend evaluation of ambient concentrations of 1,3-butadiene and chloroprene in Texas. Chemico-Biological Interactions 166: 44–51.

Sound Science

11. Grant, R.L., B.J. Kadlubar, N.K. Erraguntla, and M. Honeycutt. 2007. Evaluation of acute inhalation toxicity for chemicals with limited toxicity information. Regulatory Toxicology and Pharmacology 47: 261–73.

12. Grant, R.L., J. Haney, A.L. Curry, and M. Honeycutt. 2009. Development of a unit risk factor for 1,3-butadiene based on an updated carcinogenic toxicity assessment. Risk Analysis 29: 1726–42.

13. Grant, R.L., J. Haney, A.L. Curry, and M. Honeycutt. 2010. A chronic reference value for 1,3-butadiene based on an updated noncancer toxicity assessment. Journal of Toxicology and Environmental Health, Part B, 13: 460–75.

14. Grant, R.L., A.F. Jenkins. 2015: Use of In Vivo and In Vitro Data to Derive a Chronic Reference Value for Crotonaldehyde Based on Relative Potency to Acrolein, Journal of Toxicology and Environmental Health, Part B, DOI: 10.1080/10937404.2015.1081574.

15. Grant, R.L., S. Taiwo, and D. McCant. 2015. Assessment of chronic inhalation non-cancer toxicity for diethylamine. Inhalation Toxicology DOI: 10.3109/08958378.2015.1103338.

16. Haney, J.T. 2016. Historical drinking water contamination at Camp Lejeune: Regulatory risk assessor and personal perspectives. Human and Ecological Risk Assessment: An International Journal. Human and Ecological Risk Assessment: An International Journal 22:4, 1029-1035.

17. Haney, J.T. 2015a. Use of dose-dependent absorption into target tissues to more accurately predict cancer risk at low oral doses of hexavalent chromium. Regulatory Toxicology and Pharmacology 71: 93-100.

18. Haney, J.T. 2015b. Implications of dose-dependent target tissue absorption for linear and non-linear/threshold approaches in development of a cancer-based oral toxicity factor for hexavalent chromium. Regulatory Toxicology and Pharmacology 72: 194-201.

19. Haney, J.T., 2015c. Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium. Regulatory Toxicology and Pharmacology 73: 834-852.

20. Haney, J.T., T. Phillips, R.L. Sielken, and C. Valdez-Flores. 2015. Development of an inhalation unit risk factor for isoprene. Regulatory Toxicology and Pharmacology 73: 712-725.

21. Haney, J.T., N. Erraguntla, R.L. Sielken, et al. 2012. Development of a cancer-based chronic inhalation reference value for hexavalent chromium based on a nonlinear-threshold carcinogenic assessment. Regulatory Toxicology and Pharmacology 64: 466–80.

22. Haney, J.T., N. Erraguntla, R.L. Sielken, et al. 2014. Development of an inhalation unit risk factor for hexavalent chromium. Regulatory Toxicology and Pharmacology 68: 201–11.

Sound Science

23. Haney J, McCant D, Honeycutt M, Lange S. 2018. Development of an inhalation reference concentration for diethanolamine. Regulatory Toxicology and Pharmacology 92:55-66

24. Hofelt, C.S., M. Honeycutt, J.T. McCoy, and L.C. Haws. 2001. Development of a metabolism factor for polycyclic aromatic hydrocarbons for use in multipathway risk assessments of hazardous waste combustion facilities. Regulatory Toxicology and Pharmacology 33(1): 60–65.

25. Kirman, C.R., and R.L. Grant. 2012. Quantitative human health risk assessment for 1,3-butadiene based upon ovarian effects in rodents. Regulatory Toxicology and Pharmacology 62: 371–84.

26. McCant, D., S. Lange, J. Haney and M. Honeycutt. 2017. The perpetuation of the misconception that rats receive a 3–5 times lower lung tissue dose than humans at the same ozone concentration. Inhalation Toxicology DOI: 10.1080/08958378.2017.1323982.

27. Myers, J.L. 2016. Development of an inhalation unit risk factor for ethylene dichloride. Inhalation Toxicology 28(9): 403–409.

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29. Myers, J.L. and R.L. Grant. 2015. Development of a chronic inhalation reference value for hexamethylenediamine using an exposure model based on the dihydrochloride salt. Inhalation Toxicology 27(9): 440-449.

30. Phillips, T.D., S.E. Ethridge, and R.L. Grant. 2011. Practical Implementation of the threshold of concern and NOAEL-to-LC50 ratio factor approach to determine acute effects screening levels. The Toxicologist, Tox. Sci. (Suppl. 2), 120: Abstract No. 1523.

31. Phillips, T.D., R.L. Sielken, and C. Valdez-Flores. 2013. Development of a regulatory value for the carcinogenic potential of isoprene via inhalation exposure. The Toxicologist 132(1): Abstract No. 1565.

32. Rhomberg, L., J. Goodman, L. Bailey, R. Prueitt, N. Beck, C. Bevin, M. Honeycutt, N. Kaminski, G. Paoli, L. Pottenger, R. Scherer, K. Wise, and R. Becker. 2013. A survey of frameworks for best practices in weight-of-evidence analyses. Critical Reviews in Toxicology 43(9):753–84.

33. Shaw, B.W., Lange, S.S., and M.E. Honeycutt. 2015. “Lowering the Ozone Standard Will Not Measurably Improve Public Health.” Environmental Manager, May issue, pp. 26-31.

34. Haney, J.T., D. McCant, R.L. Sielken, C. Valdez-Flores, and R.L. Grant. 2012.Development of a unit risk factor for nickel and inorganic nickel compounds based on an updated carcinogenic toxicity assessment. Regulatory Toxicology and Pharmacology 62: 191–01.

Sound Science Objectively Reviewed

- Ontario, Canada Ministry of Environment (MOE):
  - ✓ *Deemed the assessment of 1,3-butadiene published by the TCEQ as the most scientifically-sound* after reviewing chemical assessments from Health Canada and Environment Canada, the Province of Quebec, the USEPA, the Swedish Institute of Environmental Medicine, the United Kingdom, and the World Health Organization (WHO), and the States of Louisiana, Massachusetts, Michigan, Minnesota, New Jersey, New York, Ohio, North Carolina, California, and Texas.

Sound Science Objectively Reviewed

- Peer Reviewers on USEPA's Proposed Mercury Air Toxics Standards (MATS) Rule in regard to nickel:
  - ✓"*I would recommend using the TCEQ URE*…The risk assessment leading to the derivation of this number was performed recently, included an updated and critical review of the literature, and appears to be comprehensive with an emphasis on health protection."
  - ✓"*Use the TCEQ URE*…This approach: (1) uses human data for the risk estimate, (2) takes advantage of a nickel-exposed cohort (Grimsrud 2003) for which there are data on the prevalence of smoking."
  - ✓USEPA's independent experts recommended they use our nickel URF.

Sound Science Objectively Reviewed

- The Risk Assessment Specialty Section (RASS) of the Society of Toxicology (SOT) recognized two of our 2015 papers on CrVI at the 2016 SOT conference…

Sound Science

- RASS Top 10 Risk Assessment Application Papers of 2015:

Regulatory Toxicology and Pharmacology 71 (2015) 93–100

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Regulatory Toxicology and Pharmacology

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Use of dose-dependent absorption into target tissues to more accurately predict cancer risk at low oral doses of hexavalent chromium

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Sound Science

- RASS Top 10 Risk Assessment Application Papers of 2015:

Regulatory Toxicology and Pharmacology 73 (2015) 834–852

Contents lists available at ScienceDirect

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Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium

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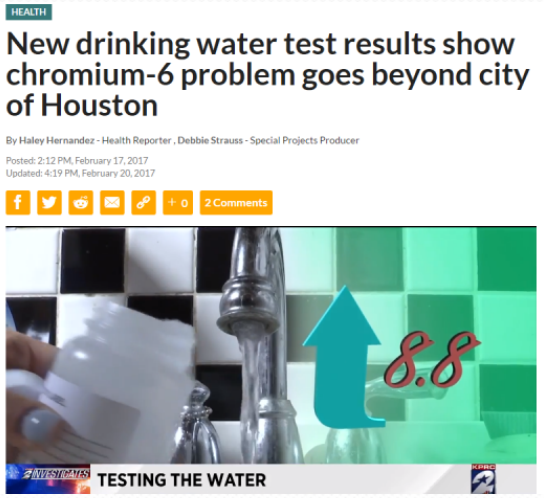
Sound Science is Needed for CrVI

- Recent work on the CrVI oral carcinogenic MOA is important and timely work.

  e.g., Does a grab sample of 8.8 ppb CrVI represent a dangerous drinking water problem?



- Some risk perspective based on best available science would be beneficial to the public here.

Sound Science is Needed for CrVI

- Over the past few years, a great deal of new research has been conducted specifically to generate data to better inform the MOA analysis for CrVI-induced carcinogenesis and to improve cross-species extrapolation (e.g., Thompson et al., 2011a, 2011b, 2012a, 2013a; Kirman et al., 2012, 2013; Proctor et al., 2012; Kopec et al., 2012a, 2012b; O'Brien et al., 2013; Suh et al., 2014; Thompson et al., 2015a, 2015c, 2017).

- Thorough evaluation of these data is essential to a better scientific understanding of the carcinogenic MOA operating in rodent studies (e.g., NTP, 2008) and CrVI toxicokinetics following oral exposure.

- This is important considering the significant regulatory challenge of extrapolating high oral dose rodent study results to environmentally-relevant human doses that are orders of magnitude lower in a meaningful, toxicologically-predictive manner.

Sound Science is Needed for CrVI

- Regulatory agencies should duly consider these data to inform key areas of the dose-response assessment such as the MOA (e.g., key events), toxicokinetics (e.g., dose-dependent differences in target tissue absorption, cross-species PBPK), and biologically-plausible expectations about potential thresholds and any low-dose risk.

CrVI Toxicokinetic Implications

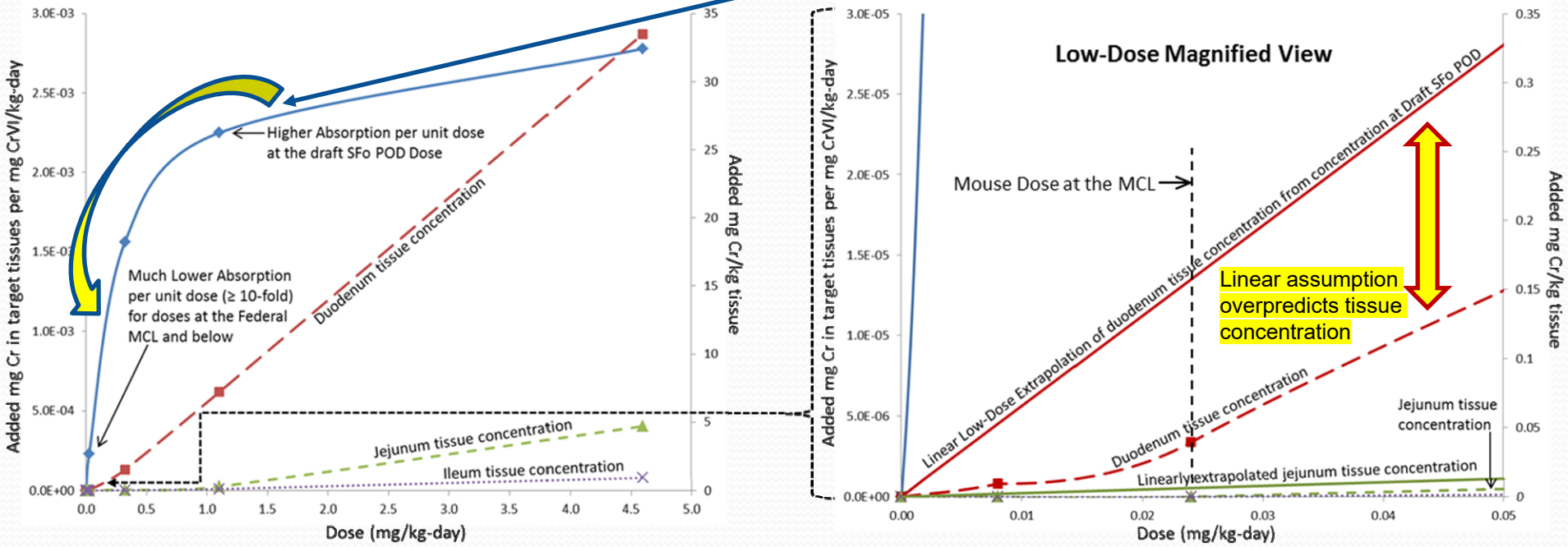
- Recent analyses of CrVI toxicokinetic (TK) data (Kirman et al., 2012) revealed *appreciable dose-dependent differences in target tissue absorption* (Haney, 2015a, 2015b).

- That is, *the dose fraction absorbed* (CrVI absorbed by target tissues per unit dose) *progressively decreases with decreasing oral dose*.

CrVI Toxicokinetic Implications

- The *relationship between oral dose and target tissue dose is non-linear* across doses of interest..

Figure 1: Dose-Dependent Changes in Mouse Target Tissue Absorption per Unit Dose and Low-Dose Nonlinearity in Absorbed Tissue Concentration versus Dose



Haney (2015c)

CrVI Toxicokinetic Implications

- Separate from MOA considerations, *any toxicity factor that assumes linearity* (e.g., between oral dose and target tissue dose or risk such as the SFo) *cannot account for the non-linear target tissue TK* resulting from the dose fraction absorbed progressively decreasing with decreasing oral dose.

CrVI Toxicokinetic Implications

- The result of non-linear TK is that even if the carc. MOA were mutagenic, *a SFo can only provide a "correct" risk estimate at the high, environmentally-irrelevant oral POD used to calculate it*.
- This issue is discussed in our first paper (Haney, 2015a), which provides a method for calculating *dose-specific SFo correction factors* based on how the dose fraction absorbed progressively decreases with oral dose (under an initial assumption of a mutagenic MOA).

CrVI Toxicokinetic Implications

- Implication of dose-dependent CrVI target tissue absorption for use of a SFo: *overestimating risk* (exacerbated more so considering the MOA data).

Figure 2: Potential Human Excess Risk versus Lower Dose Adjusted for Dose-Dependent Differences in Target Tissue Absorption

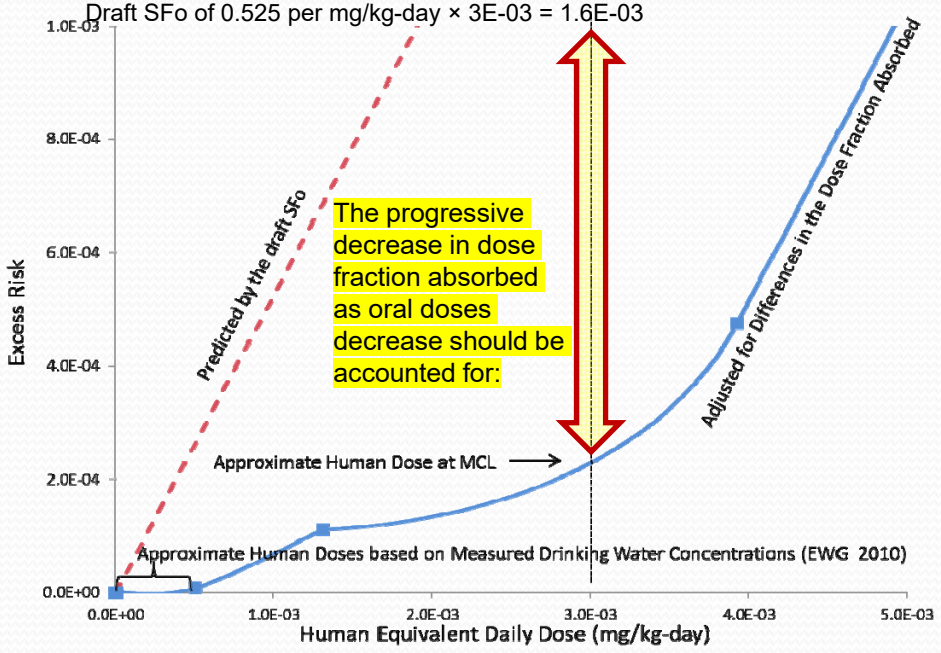
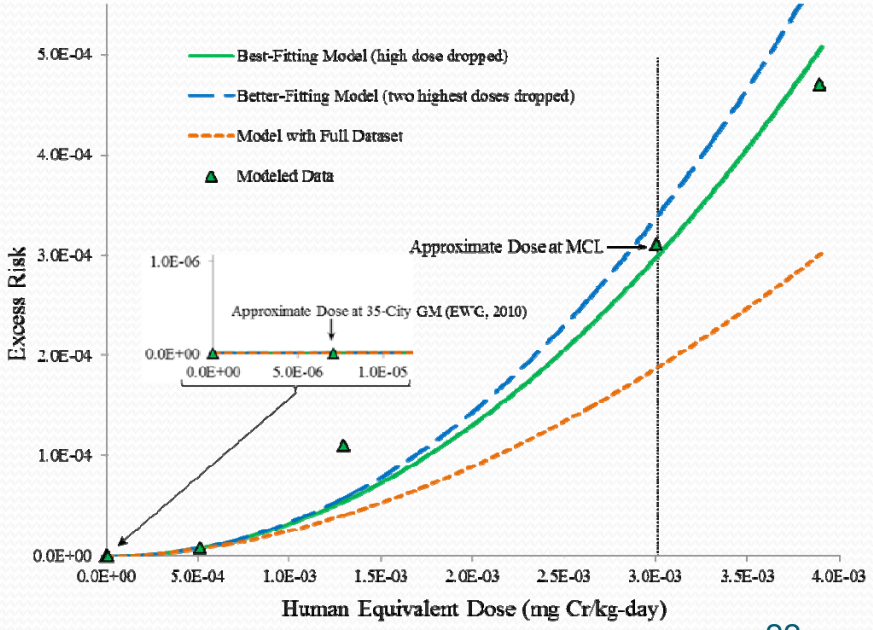


Figure 3: Non-Linear, Non-Threshold Model Fit for Potential Human Excess Risk versus Lower Dose Adjusted for Dose-Dependent Differences in Absorption



Haney (2015c)

CrVI Toxicokinetic Implications

- Thus, without consideration of the non-linear TK of progressively lower dose fraction absorption by target tissues as oral CrVI doses decrease, *it appears the simple application of an SFo progressively overestimates target tissue dose and risk at progressively lower and more environmentally-relevant doses*.

CrVI Carcinogenic MOA

- Published MOA analyses and the underlying data (e.g., McCarroll et al., 2010; Thompson et al., 2011b, 2013a) were reviewed to assess the overall weight-of-evidence (WOE) for the most scientifically-supported MOA (i.e., mutagenic versus non-mutagenic/threshold) based on currently available scientific evidence and its strength for selection of the most scientifically-defensible, non-linear approach (i.e., non-threshold versus threshold).

CrVI Carcinogenic MOA

- The WOE supporting both a non-mutagenic and mutagenic MOA was assessed in regard to:
  - ✓ Mutagenic potential pertinent to NTP study tumors;
  - ✓ CrVI-induced mutagenicity as the initiating event;
  - ✓ Dose-response concordance; and
  - ✓ Temporal concordance.

CrVI Carcinogenic MOA

- While a detailed presentation of the relevant data is challenging for a PowerPoint presentation, Table 6 below shows the progression of responses with dose…

Table 6: Summary of Dose-Response Data Relevant to the MOA

Response [a]	Drinking Water Concentration mg SDD/L					
	0.3 (0.1 mg CrVI/L)	4 (1.4 mg CrVI/L)	14 (5 mg CrVI/L)	60 (20 mg CrVI/L)	170 (60 mg CrVI/L)	520 (180 mg CrVI/L)
Cr in Duodenum (villi)	✘	✘	✓	✓	✓	✓
Oxidative Changes	✘	✘	✓	✓*	✓*	✓*
Gene Expression Changes	✘	✘	✓*	✓*	✓*	✓*
Villus Toxicity	✘	✘	✘	✓	✓*	✓*
Crypt Hyperplasia	✘	✘	✘	✓	✓	✓*
K-ras Mutations	✘	✘	✘	✘	✘	✘
Crypt MN	✘	✘	✘	✘	✘	✘
Crypt DNA Damage (γ-H2AX)	NA	✘	NA	✘	NA	✘

[a] ✓=presence of response due to 90-day exposure, with "*" denoting that 7-day exposure also induced the effect; ✘=absence of response; NA=not assessed.

Haney (2015c)

CrVI Carcinogenic MOA

- Table 5 summarizes *Key Considerations in the Carcinogenic MOA WOE…*

Table 5: Key Considerations in the Carcinogenic MOA Weight-of-Evidence

Evidence for non-mutagenic MOA [based on target tissue data following *in vivo* drinking water exposure][a]	Scientific relevance and weight[b]	Evidence for mutagenic MOA [based on non-target tissue data following various exposure scenarios/conditions][c]
Mutagenic potential pertinent to NTP study tumors (1) No increased *Kras* mutation frequency in target tissue (i.e., duodenum) due to 90-day exposure to 0.3 –520 mg SDD/L drinking water; (2) No DNA damage - negative results for MN[d], KN, AI, MI, and γ-H2AX immunostaining in duodenal crypts due to drinking water exposure for 7 and/or 90 days; and (3) Additionally, no *Apc* involvement or increased Wnt/β-catenin signaling due to 90-day drinking water exposure.	> ++ **Target tissue** – + ***In vivo*** +/– + **Relevant Study Species for SI Tumors** +/– + **Relevant Exposure Route** –/+ + **Relevant Dose(s)** +/– ++ **Drinking Water Exposure** –/+ ⇦ High hierarchy of evidence data from target tissue. ⇦ Data collected following *in vivo* exposure of the most relevant study species/strain via the most relevant route, exposure scenario, and dosing regimen (e.g., drinking water *ad libitum* and NTP study drinking water concentrations). ⇦ All target tissue data negative for mutagenicity and genotoxicity.	(1) Positive mutagenicity results *in vivo* in mouse skin, bone marrow, and liver (transgenic Muta™ mouse) by an environmentally- and physiologically-irrelevant exposure route (i.p.); (2) Generally positive genotoxicity results *in vivo* in non-target tissues of rats and mice exposed via routes/scenarios either entirely or largely irrelevant to the NTP study (e.g., i.p. and gavage as opposed to drinking water exposure that produced negative results in several 3-month studies), except for one rat drinking water study qualitatively positive for DNA-protein crosslinks by electrophoresis (negative by the well-established alkaline elution method) in the liver but negative in lymphocytes (Coogan et al., 1991); and (3) *In vitro*, generally positive results for mutagenicity/genotoxicity in non-target tissue cells and bacteria.

Relevant supporting data for non-mutagenic vs. mutagenic MOAs are presented and assigned weight.

CrVI Carcinogenic MOA

- Table 5 summarizes *Key Considerations in the Carcinogenic MOA WOE…*

CrVI-induced mutagenicity as the initiating event

(1) Same negative mutagenicity and genotoxicity evidence in target tissue as above following 7- and/or 90-day exposure; plus

(2) Signs of duodenal villous toxicity and initial signs of hyperplasia (e.g., larger crypt area) begin as early as day 8 at 170 mg SDD/L, the drinking water concentration where significant villous toxicity (e.g., 100% prevalence of atrophy/blunting) and duodenal crypt hyperplasia (i.e., prevalence, significantly increased enterocytes/crypt and crypt area) is also found later at day 91;

(3) Only one tumor location in each species (portal of entry) despite the presence of Cr in multiple tissues; and

(4) Tumors of the mouse small intestine did not occur in the 90-day NTP study or early in the 2-year study (≥451 days ♂, ≥625 days ♀) and were not associated with lethality or metastases.

>

(same +/− as above)

⇦ High hierarchy of evidence data from target tissue.

⇦ Data collected following *in vivo* exposure of the most relevant study species/strain by the most relevant route, exposure scenario, and dosing regimen (e.g., drinking water *ad libitum* and NTP study drinking water concentrations) ⇦ Data show target tissue (i.e., duodenal crypt) hyperplasia in the absence of mutagenicity (and genotoxicity), but in the presence of significant villous toxicity (e.g., prevalent atrophy/blunting), in addition to characteristics not indicative of a mutagenic MOA.

(1) Same non-target tissue data as above; plus

(2) DNA adducts on pSP189 plasmids transfected into human fibroblasts immortalized with the SV virus and then transfected into *E. Coli* MBL50, which increased mutation frequency; but

(3) Primarily, DNA strand breaks in the leukocytes of mice exposed via oral gavage (Danadevi et al., 2001), and secondarily DNA-protein crosslinks as evaluated qualitatively by electrophoresis (but negative by the well-established alkaline elution method) in the liver (but not in the lymphocytes) of rats exposed via drinking water (Coogan et al., 1991), neither of which are mutations or in target tissue.

CrVI Carcinogenic MOA

- Table 5 summarizes *Key Considerations in the Carcinogenic MOA WOE…*

Dose–response concordance	>	To demonstrate dose–response concordance between the key mutational event initiating the carcinogenic process in the mouse small intestine and subsequent events in the hypothesized mutagenic MOA, the McCarroll et al. (2010) analysis hinges upon:
(1) Increased duodenum tissue concentrations of Cr at ≥14 mg SDD/L for 90 days;	++ **Target tissue** –	(1) Primarily, DNA strand breaks in Swiss mouse leukocytes (a non-mutation endpoint, not in a tissue susceptible to CrVI-induced carcinogenesis) due to single oral gavage at a cited dose of 0.6 mg/kg[e] (Danadevi et al., 2001); and
(2) Redox changes (GSH/GSSG) in the duodenum at ≥60 mg SDD/L for 7 days and ≥14 mg SDD/L for 90 days, with gene expression changes indicative of oxidative stress at ≥60 mg SDD/L for 90 days;	+ ***In vivo*** +	(2) Secondarily, DNA-protein cross-links in the rat liver (another non-mutation endpoint not in target tissue) as assessed qualitatively by electrophoresis (negative by the well-established alkaline elution method) at ≈6–9 mg CrVI/kg-day for 3 weeks via drinking water (Coogan et al., 1991).[f]
(3) Signs of duodenal villous toxicity and initial signs of hyperplasia (e.g., larger crypt area) beginning at 170 mg SDD/L for 7 days, and 60 mg SDD/L for 90 days (e.g., 40% prevalence of villous atrophy/blunting, 30% prevalence of crypt hyperplasia);	+ **Relevant Study Species for SI Tumors** +/–	
(4) Increased villous toxicity and significant crypt hyperplasia at 520 mg SDD/L for 7 days, with increased villous toxicity (e.g., atrophy/blunting) and duodenal crypt hyperplasia (i.e., prevalence, significantly increased enterocytes/crypt and crypt area) beginning at 170 mg SDD/L for 90 days;	+ **Relevant Exposure Route and Dose(s)** +	
(5) Similar but increased villous toxicity and duodenal crypt hyperplasia prevalence with further increased enterocytes/crypt and crypt area at 520 mg SDD/L for 90 days;	++ **Relevant Exposure Scenario** –/+	
(6) Qualitatively similar results for villous toxicity and crypt hyperplasia for the 2-year NTP study.	⇦ High hierarchy of evidence data from target tissue.	
(7) Mutagenicity/genotoxicity is not induced by these drinking water concentrations - no evidence of increased *Kras* mutations, crypt cytogenetic damage (i.e., negative results for MN[d], KN, AI, MI, and γ-H2AX immunostaining), *Apc* involvement or increased Wnt/β-catenin signaling at tumorigenic doses via drinking water for 7 and/or 90 days; and	⇦ Data collected following *in vivo* exposure of the most relevant study species/strain by the most relevant route, exposure scenario, and dosing regimen (e.g., drinking water *ad libitum* and NTP study drinking water concentrations).	
(8) Late onset tumorigenesis is induced at the same drinking water concentrations (60–520 mg SDD/L) as these non-mutagenic events.	⇦ Data are indicative of: (1) Increased tissue concentrations of Cr and redox changes at lower concentrations than those inducing villous toxicity and crypt hyperplasia at the same time point; (2) Significant crypt hyperplasia (60% prevalence) at 520 mg SDD/L on day 8 in the presence of significant villous toxicity (e.g., 60% prevalence of atrophy) and signs of villous toxicity and initial signs of hyperplasia that began at the next lowest dose of 170 mg SDD/L on day 8; (3) Significant crypt hyperplasia (30% prevalence) in the presence of villous toxicity (e.g., 40% prevalence of atrophy/blunting) beginning at 60 mg SDD/L on day 91 that is progressively more prevalent at 170 and 520 mg SDD/L in the presence of increased and significant villous toxicity and in the absence of crypt mutagenicity/genotoxicity; and (4) Qualitatively similar results for villous toxicity and crypt hyperplasia in the 2-year study.	
	⇦ Duodenal tumors occur at these same doses.	

CrVI Carcinogenic MOA

- Table 5 summarizes *Key Considerations in the Carcinogenic MOA WOE…*

Table 5: (continued)

Evidence for non-mutagenic MOA [based on target tissue data following *in vivo* drinking water exposure][a]	Scientific relevance and weight[b]	Evidence for mutagenic MOA [based on non-target tissue data following various exposure scenarios/conditions][c]
Temporal concordance Day 8 • Decreased GSH/GSSG ratio • Nrf2 activation/oxidative stress • Significantly increased Cr content in duodenal villi • Signs of duodenal villous toxicity (e.g., cytoplasmic vacuolization, atrophy) and initial signs of hyperplasia (e.g., larger crypt area) beginning at 170 mg SDD/L • Absence of significantly increased Cr content in duodenal crypts • Absence of aberrant nuclei (e.g., MN[d], KN) or γ-H2AX immunostaining in duodenal crypts • No *Apc* or Wnt/β-catenin changes • Transcript changes consistent with non-mutagenic MOA • Increased signs of villous toxicity and incidence of crypt hyperplasia at 520 mg SDD/L Day 91 • Decreased GSH/GSSG ratio • Nrf2 activation • Significantly increased Cr content in duodenal villi • Increased γ-H2AX immunostaining in duodenal villi in the absence of aberrant villous foci indicative of transformation • Diffuse hyperplasia at ≥62.5 mg SDD/L • Significant duodenal villous toxicity (e.g., atrophy/blunting) and crypt hyperplasia beginning at 60 mg SDD/L • Absence of significantly increased Cr content in duodenal crypts • Absence of aberrant nuclei or γ-H2AX immunostaining in duodenal crypts • No change in *Kras* mutation • No *Apc* or Wnt/β-catenin changes • Increased and significant villous toxicity and duodenal crypt hyperplasia (i.e., prevalence, significantly increased enterocytes/crypt and crypt area) at 170 and 520 mg SDD/L 2-Year (NTP Study) • Qualitatively similar results for villous toxicity and crypt hyperplasia • Adenomas (≥451 days ♂, ≥693days ♀) • Carcinomas (≥729 days ♂, ≥625 days♀)	> ++ **Target tissue** – + ***In vivo*** + + **Relevant Study Species for SI Tumors** +/– + **Relevant Exposure Route and Dose(s)** + ++ **Relevant Exposure Scenario** –/+ ⇦ High hierarchy of evidence data from target tissue. ⇦ Data collected following *in vivo* exposure by the most relevant route, exposure scenario, and dosing regimen (e.g., drinking water *ad libitum* and NTP study drinking water concentrations). ⇦ Data are indicative of: (1) Signs of villous toxicity and initial signs of crypt hyperplasia on day 8 at 170 mg SDD/L, with increased villous toxicity and significant crypt hyperplasia on day 91 at the same dose; (2) Significant villous toxicity and crypt hyperplasia on day 8 at 520 mg SDD/L, with further increased villous toxicity and significant hyperplasia (e.g., prevalence, crypt area) on day 91 at the same dose (in the absence of crypt mutagenicity/genotoxicity); and (3) For the 2-year study, qualitatively similar results for villous toxicity and crypt hyperplasia. ⇦ Temporally, these effects occurring as early as day 8 significantly precede the late onset duodenal tumors (e.g., adenomas at ≥451 days).	To demonstrate temporal concordance between key events in the hypothesized mutagenic MOA for the carcinogenic process in the mouse small intestine, the McCarroll et al. analysis again hinges upon: • Primarily, DNA strand breaks in Swiss mouse leukocytes at day 1 due to single oral gavage at a dose of 0.6 mg/kg[e] (Danadevi et al., 2001); • Secondarily, after 3 week exposure to ≈6–9 mg CrVI/kg-day via drinking water (≈day 21), DNA-protein crosslinks in the rat liver (another non-mutation endpoint not in a tissue susceptible to CrVI-induced carcinogenesis) evaluated qualitatively by electrophoresis (but negative by the well-established alkaline elution method) (Coogan et al., 1991)[f]; plus • Cell proliferation (hyperplasia) at day ≥90.

Please see the associated open-access publication for a full, *ad nauseam* discussion (Haney, 2015c).

CrVI Carcinogenic MOA

- Bottom Line: *Detailed review of available data indicates that a non-mutagenic MOA is best supported by the scientific evidence*.

- Data from the CrVI MOA research project were collected specifically to be directly relevant for informing the MOA.

CrVI Carcinogenic MOA

- The supporting target tissue data are highest on the scientific evidence hierarchy for evaluating the likely MOA, especially considering they were collected following *in vivo* exposure of the most relevant study species/strain for CrVI-induced tumors of the small intestine via the most relevant exposure route, exposure scenario, and dosing regimen (e.g., drinking water *ad libitum* and NTP study drinking water concentrations).

CrVI Carcinogenic MOA

- Much weaker evidence for a mutagenic MOA relies on an assortment of non-target tissue data collected following various exposure scenarios/ conditions, not specifically for MOA analysis.

CrVI Carcinogenic MOA

- The WOE indicates *cytotoxicity-induced regenerative hyperplasia is the most scientifically well-supported MOA* (e.g., consistent with Thompson et al., 2013a and Health Canada, 2015).

- Health Canada (2015) concurs, indicating that the evidence for a mutagenic MOA is weak, and confidence in a cytotoxic MOA is high.

CrVI Carcinogenic MOA

- Compensatory crypt enterocyte hyperplasia induced by chronic villous toxicity should be considered as required (not always sufficient) for CrVI-induced intestinal tumorigenesis.

- That is, *cytotoxicity-induced regenerative hyperplasia should be considered a key event in the carcinogenic MOA for oral exposure to CrVI.*

- Consequently, the threshold (i.e., RfD) approach should be adopted for assessing the potential intestinal carcinogenicity of oral exposure to CrVI.

CrVI Carcinogenicity-Based RfD

- The RfD was developed using standard dose-response assessment methodologies and USEPA BMD software.

- Diffuse hyperplasia was utilized as the key precursor event based on the MOA analysis.

CrVI Carcinogenicity-Based RfD

- Based on NTP (2008) data, diffuse hyperplasia only has a strong, well-defined dose-response relationship in the mouse duodenum (see Appendices C and D of NTP, 2008).

- This is consistent with both significant tissue absorption of CrVI by the duodenum and the duodenum as the most tumorigenically-responsive tissue.

- Therefore, the duodenum was selected as the critical mouse target tissue for BMD analysis.

CrVI Carcinogenicity-Based RfD

- The incidence of diffuse hyperplasia in the duodenum of female mice was used for BMD modeling since:
  - Statistical analyses did not reveal differences between male and female mice in hyperplastic or tumorigenic response to CrVI exposure (Thompson et al., 2013b);
  - The dose-response for diffuse hyperplasia in female mice is strong and more monotonic than that in male mice (see Tables C4 and D4 of NTP, 2008); and
  - Importantly, the water concentrations used in NTP (2008) for female mice correspond to those used in Kirman et al. (2012) to determine added Cr concentrations in mouse target tissues due to CrVI oral exposure, which is a very useful internal dose metric for BMD modeling.

CrVI Carcinogenicity-Based RfD

- Accordingly, the incidence of diffuse hyperplasia in the duodenum of female mice from NTP (2008) along with the duodenum tissue concentrations (added mg Cr/kg tissue) reported in Kirman et al. (2012) were used for BMD modeling in the principal analysis.

Table 2: Added Chromium Duodenum Concentrations and Diffuse Hyperplasia in B6C3F1 Mice

Drinking Water Concentration (mg SDD/L)	**Duodenum Tissue Concentration** (mean added mg Cr/kg tissue)	**± SD**	**95% UCL** (added mg Cr/kg tissue)	**95% LCL** (added mg Cr/kg tissue)	**Number of Animals (n)**	**Diffuse Hyperplasia (# animals)**
0	0	---	---	---	50	0
14	7.2	0.8	7.8	6.6	50	16
60	33.5	5.0	37.2	29.8	50	35
170	42.4	12.4	51.5	33.3	50	31
520	60.9	14.1	71.3	50.5	50	42

Haney (2015c)

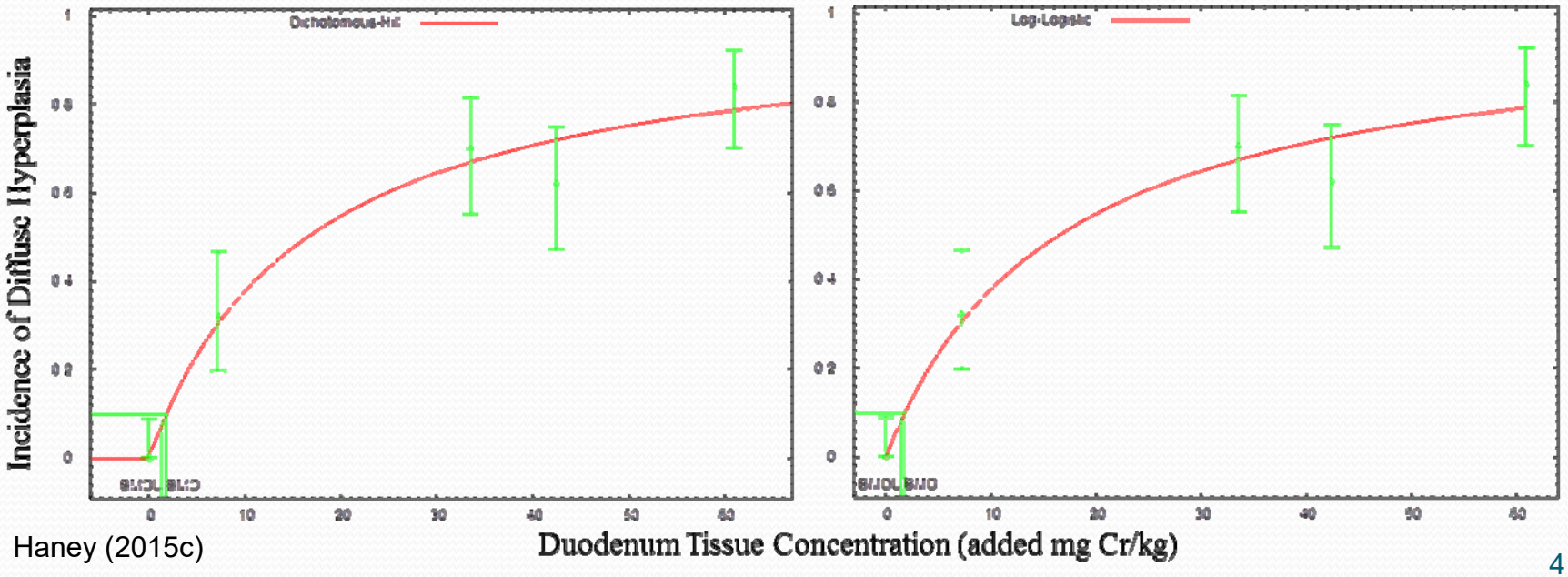
CrVI Carcinogenicity-Based RfD

- A benchmark response (BMR) of 10% was used so that the BMD and 95% lower confidence limit on the BMD (BMDL) would be calculated at a BMR that does not extrapolate farther than necessary below the range of the data.

CrVI Carcinogenicity-Based RfD

- The Log-Logistic and Dichotomous-Hill models provided adequate and almost identical fits to the mouse data (Table 2) with a goodness-of-fit p value >0.1, lowest AIC, and scaled residuals <|2|.

Figure 4: Diffuse Hyperplasia Incidence versus Duodenum Tissue Concentration



Haney (2015c)

CrVI Carcinogenicity-Based RfD

- The mouse BMD10 value was 1.83 added mg Cr/kg tissue for both models using mean added mg Cr/kg tissue as the internal dose metric.

- The average mouse BMDL10 of 1.39 added mg Cr/kg tissue (based on individual model values of 1.37 and 1.41 added mg Cr/kg tissue) was used as the POD for diffuse hyperplasia in the duodenum for the derivation of an RfD.

CrVI Carcinogenicity-Based RfD

- The mouse POD of 1.39 added mg Cr/kg duodenum tissue was converted to a corresponding oral dose based on the relationship between duodenum tissue concentration (mean mg Cr/kg tissue) and oral dose (mg/kg-day) that was modeled in Haney (2015a).

- This POD falls between two of the tissue concentrations modeled and is similar to one of the modeled concentrations (1.5 mg Cr/kg tissue) where the estimated and observed values show excellent agreement (i.e., the scaled residual is 0.421, well below |2|), which increases confidence in the estimate at the POD.

CrVI Carcinogenicity-Based RfD

- A mouse oral dose of 0.31 mg CrVI/kg-day is estimated to correspond to the POD duodenum tissue concentration.

Table 4: Duodenum Best-Fitting Model Tissue Concentration Prediction

Hill Model (non-constant variance) Equation:
Y [tissue conc. in mg Cr/kg at dose] = intercept + v*dose^n/(k^n + dose^n)

Parameters	Inputs
Oral Dose (mg/kg-day)	**0.31 (oral POD)**
intercept	0.018
v	62.397
n	1.406
k	4.638
Y [tissue conc. in mg Cr/kg at dose]	**1.39 ($BMDL_{10}$)**

Haney (2015c)

CrVI Carcinogenicity-Based RfD

- Without TCEQ having PBPK models for cross-species extrapolation, interestingly, the ultimate application of an animal-to-human uncertainty factor of 10 to this mouse POD results in a value (0.031 mg/kg-day) that is:
  - Below the lower end of the range of average human equivalent doses (HED values of 0.05-0.1 mg/kg-day) cited in a recent USEPA CrVI PBPK study;
  - *Practically identical to the more conservative HED of 0.028 mg/kg-day (pH=5) based on a similar evaluation (e.g., using the BMDL10 for diffuse epithelial hyperplasia) in USEPA's Sasso and Schlosser (2015) paper*; and
  - 4.5-fold lower than the HED of 0.14 mg/kg-day (pH=2.5) based on the similar evaluation (see Table 1 of Sasso and Schlosser 2015).

CrVI Carcinogenicity-Based RfD

- Dividing the mouse oral dose of 0.31 mg CrVI/kg-day by the same uncertainty factors (UF_A=10, UF_H=10, UF_D=1) as used in USEPA (2010) results in an *RfD of 0.0031 mg CrVI/kg-day*.

- This value is the same as USEPA's IRIS CrVI RfD (0.003 mg CrVI/kg-day) and shows remarkable agreement with more current values derived through different methods, increasing confidence:
  - RfDs published recently (0.003-0.006 mg CrVI/kg-day; Thompson et al. 2013, 2017); and
  - Health Canada's 2015 draft TDI (0.0044 mg CrVI/kg-day).

CrVI Carcinogenicity-Based RfD

- Based on MOA analysis, the TCEQ considers its carcinogenicity-based RfD protective of the potential carcinogenic effects of oral exposure to CrVI.

In Conclusion

- The TCEQ's goal is to use sound science in deriving toxicity factors.

- Based on analysis of the MOA data, in 2016 the TCEQ finalized an RfD (0.0031 mg CrVI/kg-day) to protect against the potential carcinogenic effects of oral CrVI exposure by protecting against cytotoxicity-induced regenerative hyperplasia as a key precursor event in the oral carcinogenic MOA.

In Conclusion

- This and similar assessments have important regulatory, public health and risk assessment/communication implications (e.g., whether environmental exposures such as typical drinking water concentrations represent a realistic health concern or not).

- For example, this RfD happens to correspond to the approximate human intake at the federal total chromium MCL (0.1 mg/L), lending support for its health protectiveness even if all chromium is present as CrVI.

In Conclusion

- Lastly, I encourage you to not only read our CrVI papers, but most of all to review all the relevant data for yourselves.

- This is both time consuming and necessary to formulate your own independent scientific judgments and convictions about the WOE for the most scientifically-supported carcinogenic MOA and its strength.

- Following your independent and objective review of the data, I think you'll end up at the same MOA and low-dose extrapolation approach conclusions as the TCEQ, Health Canada, and others (i.e., non-mutagenic MOA WOE, threshold approach most scientifically defensible).

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Thank You!