Development Support Document
Proposed, June 2016

Hexavalent Chromium
Oral Reference Dose

CAS Registry Number: 18540-29-9

Prepared by

Joseph T. Haney, Jr., M.S.

Toxicology Division

Office of the Executive Director

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

Executive Summary

Background

In recent years, a great deal of new research has been conducted specifically to generate data to better inform the mode of action (MOA) analysis for hexavalent chromium-induced carcinogenesis due to chronic oral exposure and to improve the extrapolation of rodent oral study results to humans (e.g., Thompson et al. 2011a, 2011b, 2012a, 2012b, 2012c, 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, 2015b, 2015c). These research project data have been thoroughly evaluated to gain critical scientific understanding and insight into key areas of carcinogenic dose-response assessment for hexavalent chromium (CrVI) such as:

- The carcinogenic MOA (i.e., key events) operating in relevant rodent studies (e.g., NTP 2008);
- CrVI toxicokinetics following oral exposure (e.g., dose-dependent differences in target tissue absorption); and
- Data-informed, biologically-plausible expectations about potential low-dose risk.

The current, significantly greater scientific understanding of these issues (especially considering the previous lack of sufficient relevant data and understanding just 5-6 years ago) is of paramount importance considering the substantial regulatory challenge of extrapolating high oral dose results from laboratory animal studies to environmentally-relevant human doses that are orders of magnitude lower in a meaningful, toxicologically-predictive manner (e.g., the mouse dose at the lowest water concentration used in NTP 2008 is about 74,000 times higher than the approximate human dose corresponding to the 35-city geometric mean drinking water concentration reported in EWG 2010).

TCEQ Scientific, Peer-Reviewed Publications

In addition to the numerous studies published as part of the CrVI MOA research project, TCEQ staff have independently and critically evaluated the relevant published study data (and supplemental data) and its implications for the carcinogenic dose-response assessment of oral exposure to CrVI. As part of that scientific endeavor, three manuscripts have been published in a scientific peer-reviewed journal (Haney 2015a, 2015b, 2015c). The last of these scientific, open-access articles (Haney 2015c) considers both the non-linear, non-threshold approach as well as the threshold (i.e., reference dose) approach prior to conducting a weight-of-evidence (WOE) analysis of available MOA data. The WOE indicates that cytotoxicity-induced regenerative hyperplasia is indubitably the most scientifically well-supported MOA. Health Canada (2015) concurs that confidence in a cytotoxic MOA is high (and evidence for a mutagenic MOA is weak). More specifically, compensatory crypt enterocyte hyperplasia induced by chronic villous toxicity should be considered as a required (but not always sufficient) key event in CrVI-induced intestinal tumorigenesis. Consequently, the reference dose (RfD) approach is the most

scientifically-defensible approach based on the WOE of available MOA information and analyses conducted for the most scientifically-supported MOA and should be adopted for assessing the potential intestinal carcinogenicity of oral exposure to CrVI (Haney 2015c).

RfD Derivation

The RfD derived in Haney (2015c) will be adopted by the TCEQ. The RfD of 0.0031 mg CrVI/kg-day was derived to protect against cytotoxicity-induced regenerative hyperplasia as a key precursor carcinogenic MOA event. Briefly, the duodenum was selected as the critical mouse target tissue for benchmark dose (BMD) analysis since diffuse hyperplasia has a strong, well-defined dose-response relationship in the mouse duodenum. This is consistent with both significant tissue absorption of CrVI by the duodenum and the duodenum as the most tumorigenically responsive tissue. The incidence of diffuse hyperplasia in the duodenum of female mice was used for BMD modeling since: (1) Statistical analyses did not reveal differences between male and female mice in hyperplastic or tumorigenic response to CrVI exposure (Thompson et al. 2013b); (2) 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 (3) Importantly, the water concentrations used in NTP (2008) for female mice correspond to those used in Kirman et al. (2012) to determine added chromium (Cr) concentrations in mouse target tissues due to CrVI oral exposure, which is a useful internal dose metric for BMD modeling. 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. 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 did not extrapolate farther than necessary below the range of the data.

The Log-Logistic and Dichotomous-Hill models provided adequate and almost identical fits to the mouse data with a goodness-of-fit p value >0.1, lowest Akaike Information Criterion, and scaled residuals <|2|. The mouse BMD_{10} 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 $BMDL_{10}$ 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 point of departure (POD) for diffuse hyperplasia in the duodenum for derivation of the 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 CrVI/kg-day) that was modeled previously (Haney 2015a). The 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 showed excellent agreement (i.e., the scaled residual was 0.421, well below |2|), which increases confidence in the oral exposure estimate corresponding to the target tissue dose POD. A mouse oral dose of 0.31 mg CrVI/kg-day was estimated to correspond to the POD duodenum tissue concentration. Note that the application of an animal-to-human uncertainty factor (UF_A) to this mouse POD

ultimately 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 0.05-0.1mg/kg-day) cited in a recent USEPA CrVI PBPK study (Sasso and Schlosser 2015), practically identical to the more conservative HED of 0.028 mg/kg-day (pH = 5) based on a similar evaluation (e.g., using the $BMDL_{10}$ for diffuse epithelial hyperplasia), and is 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).

Dividing this mouse oral POD (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.

RfD = 3.1E-03 mg CrVI/kg-day

Comparison of TCEQ RfD with Other Published RfDs

The TCEQ-derived RfD is somewhat more conservative than, but shows remarkable agreement with, a previously published RfD (0.006 mg CrVI/kg-day; Thompson et al. 2013b) as well as Health Canada's Tolerable Daily Intake (TDI of 0.0044 mg CrVI/kg-day; Health Canada 2015). It also happens to correspond to the approximate human dose at the federal MCL for Cr (e.g., 0.1 mg/L × 2.5 L/day × 1/80 kg = 0.0031 mg/kg-day), and is considered protective of both the potential carcinogenic and noncarcinogenic effects of oral CrVI exposure. Additional details pertaining to the RfD derivation may be found in the open access article (Haney 2015c).

References

Environmental Working Group (EWG). 2010. Chromium-6 in US tap water. Environmental Working Group, December 20, 2010.

Haney J. 2015a. Use of dose-dependent absorption into target tissues to more accurately predict cancer risk at low oral doses of hexavalent chromium. Regul Toxicol Pharmacol 71, 93-100.

Haney J. 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. Regul Toxicol Pharmacol 72, 194-201.

Haney J. 2015c. Consideration of non-linear, non-threshold and threshold approaches for assessing the carcinogenicity of oral exposure to hexavalent chromium. Regul Toxicol Pharmacol 73, 834-852. Available at: http://www.sciencedirect.com/science/article/pii/S0273230015300957.

Health Canada. 2015. Chromium in drinking water (document for public consultation). Prepared by the Federal-Provincial-Territorial Committee on Drinking Water, Health Canada.

Kirman C, Hays S, Aylward L, et al. 2012. Physiologically based pharmacokinetic model for rats and mice orally exposed to chromium. Chem Biol Interact 200, 45-64.

Kirman C, Aylward L, Suh M, et al. 2013. Physiologically based pharmacokinetic model for humans orally exposed to chromium. Chem Biol Interact 204, 13-27.

Kopec A, Kim S, Forgacs A, et al. 2012a. Genome-wide gene expression effects in B6C3F1 mouse intestinal epithelia following 7 and 90 days of exposure to hexavalent chromium in drinking water. Toxicol Appl Pharmacol 259, 13-26.

Kopec A, Thompson C, Kim S, et al. 2012b. Comparative toxicogenomic analysis of oral Cr(VI) exposure effects in rat and mouse small intestinal epithelium. Toxicol Appl Pharmacol 262, 124-138.

National Toxicology Program (NTP). 2008. National Toxicology Program technical report on the toxicology and carcinogenesis studies of sodium dichromate dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies). NTP Toxicity Report 546. NIH Publication No. 08-5887.

O'Brien T, Ding H, Suh M, et al. 2013. Assessment of K-Ras mutant frequency and micronucleus incidence in the mouse duodenum following 90-days of exposure to Cr(VI) in drinking water. Mutat Res 754, 15-21.

Proctor D, Suh M, Aylward L, et al. 2012. Hexavalent chromium reduction kinetics in rodent stomach contents. Chemosphere 89, 487-493.

Sasso A, and Schlosser P. 2015. An evaluation of in vivo models for toxicokinetics of hexavalent chromium in the stomach. Toxicol Appl Pharmacol 287, 293-298.

Suh M, Thompson C, Kirman C, et al. 2014. High concentrations of hexavalent chromium in drinking water alter iron homeostasis in F344 rats and B6C3F1 mice. Food Chem Toxicol 65, 381-388.

Thompson C, Proctor D, Haws L, et al. 2011a. Investigation of the mode of action underlying the tumorigenic response induced in B6C3F1 mice exposed orally to hexavalent chromium. Toxicol Sci 123, 58-70.

Thompson C, Haws L, Harris M, et al. 2011b. Application of the US EPA mode of action framework for purposes of guiding future research: a case study involving the oral carcinogenicity of hexavalent chromium. Toxicol Sci 119, 20-40.

Thompson C, Proctor D, Suh M, et al. 2012a. Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action. Toxicol Sci 125, 79-90.

Thompson C, Hixon J, Proctor D, et al. 2012b. Assessment of genotoxic potential of Cr(VI) in the mouse duodenum: an in silico comparison with mutagenic and nonmutagenic carcinogens across tissues. Regul Toxicol Pharmacol 64, 68-76.

Thompson C, Fedorov Y, Brown D, et al. 2012c. Assessment of Cr(VI)-induced cytotoxicity and genotoxicity using high content analysis. PLoS One, 7, e42720.

Thompson C, Proctor D, Suh M, et al. 2013a. Assessment of the mode of action underlying development of rodent small intestinal tumors following oral exposure to hexavalent and relevance to humans. Crit Rev Toxicol 43, 244-274.

Thompson C, Kirman C, Proctor D, et al. 2013b. A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. J Appl Toxicol 34, 525-536.

Thompson C, Seiter J, Chappell M, et al. 2015a. Synchrotron-based imaging of chromium and gamma-H2AX immunostainingin the duodenum following repeated exposure to Cr(VI) in drinking water. Toxicol Sci 143, 16-25.

Thompson C, Young R, Suh M, et al. 2015b. Assessment of the mutagenic potential of Cr(VI) in the oral mucosa of big blue® transgenic f344 rats. Environ Mol Mutagen 56, 621-628.

Thompson C, Wolf J, Elbekai R, et al. 2015c. Duodenal crypt health following exposure to Cr(VI): Micronucleus scoring, γ-H2AX immunostaining, and synchrotron X-ray fluorescence microscopy. Mutat Res: Genet Toxicol Environ Mutagen 789-790, 61-66.

United States Environmental Protection Agency (USEPA). 2010. Toxicological Review of Hexavalent Chromium In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-10/004A. US Environmental Protection Agency, Washington, DC.