Texas Commission on Environmental Quality (TCEQ)

Responses to Public Comments

September 23, 2016

Hexavalent Chromium Oral Reference Dose

Development Support Document

The public comment period on the Development Support Document (DSD) for the proposed hexavalent chromium oral reference dose (RfD) ended September 20, 2016. The American Chemistry Council (ACC) and ToxStrategies, Inc. submitted comments on the proposed DSD. The Texas Commission on Environmental Quality (TCEQ) appreciates the effort put forth to provide comments on this proposed DSD for a hexavalent chromium RfD. The goal of the Toxicology Division and TCEQ is to protect human health and welfare based on the most scientifically-defensible approaches possible (as documented in the DSD), and evaluation of these comments furthered that goal. Comments were divided into sections and are provided below, followed by TCEQ responses.

ToxStrategies, Inc.

Comment No. 1:

The Texas Commission on Environmental Quality (TCEQ) has proposed an oral reference dose (RfD) that is protective against both non-cancerous and cancerous effects of hexavalent chromium [Cr(VI)] in drinking water. The derivation of the proposed RfD of 0.0031 mg Cr(VI)/kg-day relies on 1) evidence for a non-mutagenic threshold-based mode of action (MOA) for intestinal tumors observed in mice, 2) target tissue pharmacokinetic data, and 3) the application of conservative uncertainty factors. Each of these elements are discussed in turn below. In summary, it is our opinion that the available science fully support the TCEQ's proposed RfD of 0.0031 mg Cr(VI)/kg-day.

TCEQ Response:

The TCEQ acknowledges the comment's concurrence with the weight of the significant scientific evidence supporting the scientific defensibility of the RfD.

Comment No. 2:

<u>Selection of a non-mutagenic Mode of Action</u>

In approximately 2010, a research effort was started with the primary objective to understand the MOA for tumors observed in the 2-year cancer bioassay of Cr(VI) (NTP, 2008), and to provide important information relevant for conducting a risk assessment of oral exposure to Cr(VI) in drinking water. The Cr(VI) MOA Research (www.cr6study.info) has resulted in numerous peer-

reviewed publications in the field of toxicology that together provide support for TCEQ's use of a non-mutagenic MOA involving cytotoxicity to intestinal villi and crypt regenerative hyperplasia. At high exposure concentrations, cytotoxicity and crypt hyperplasia occur after only 7 days of exposure, which indicates that mice in the high dose groups of the NTP (2008) cancer bioassay experienced a lifetime of increased cell proliferation. Increased cell proliferation is a recognized risk factor for carcinogenesis (Cohen and Ellwein, 1990; Tomasetti and Vogelstein, 2015). The proposed MOA for Cr(VI) is also consistent with accepted nonmutagenic MOAs for other intestinal carcinogens (e.g. captan) (Gordon, 2007).

TCEQ Response:

The TCEQ acknowledges the comment's concurrence with the weight of the scientific evidence supporting use of a non-mutagenic carcinogenic mode of action (MOA) and an RfD approach protective against carcinogenic effects.

Comment No. 3:

Use of Pharmacokinetic Data

As part of the Cr(VI) MOA Research, pharmacokinetic data were collected on the rate and capacity for rodent and human gastric fluid to reduce Cr(VI) to inert Cr(III) in the stomach before transiting to the small intestine (De Flora *et al.*, 2016; Kirman *et al.*, 2012; Kirman *et al.*, 2016; Proctor *et al.*, 2012). In addition, chromium levels were measured in various tissues (including the small intestine) after 90 days of exposure to Cr(VI). These pharmacokinetic data provide an alternative (and in this case superior) dose metric to the mg/kg bodyweight dose metric employed in many risk assessments. Thus, TCEQ's use of these data for quantitative dose-response modeling and RfD derivation represents a robust approach for setting safety standards.

TCEQ Response:

The TCEQ acknowledges the comment's concurrence with target tissue dose as an internal dose metric that is superior to oral CrVI dose (mg/kg-day) for dose-response modeling, particularly considering dose-dependent differences in CrVI absorption.

Comment No. 4:

Application of Uncertainty Factors

With regard to the uncertainty factor (UF) values TCEQ applied in the derivation of the RfD, the UFs selected were consistent with those used by the U.S. EPA in their 2010 draft risk assessment (U.S. EPA, 2010). Specifically, the duration and database UFs were both 1, due to the use of chronic exposure data from the NTP (2008) 2-year cancer bioassay and adequate database on the oral toxicity of Cr(VI) (U.S. EPA, 2010). The interspecies and intraspecies UFs were each 10-fold, resulting in the same 100-fold composite UF that the U.S. EPA applied back in 2010 when less was known about the oral toxicity and carcinogenicity of Cr(VI). Although the TCEQ might have used available PBPK models for Cr(VI) to extrapolate internal dose metrics to humans (Kirman *et al.*, 2013; Kirman *et al.*, 2012) and thus potentially have reduced the total uncertainty in their

assessment, the proposed RfD is conservative and well supported. However, it is conceivable that the use of such PBPK models might result in higher RfD values (Thompson *et al.*, 2014).

TCEQ Response:

The TCEQ acknowledges that the RfD is conservative. For example, because TCEQ's ultimate mouse point of departure oral dose of 0.31 mg CrVI/kg-day divided by the animal-to-human uncertainty factor (UF_A of 10) results in a value (0.031 mg/kg-day) that is practically identical to the lowest human equivalent dose (0.028 mg/kg-day; pH = 5; $BMDL_{10}$ for diffuse epithelial hyperplasia) in recent physiologically-based pharmacokinetic (PBPK) evaluations by the U.S. Environmental Protection Agency (USEPA) considering toxicokinetic variability (Sasso and Schlosser 2015), there was potential scientific justification for a lower intraspecies UF based primarily on the remaining uncertainty in intraspecies toxicodynamics. However, the TCEQ conservatively utilized a full UF_H of 10.

Comment No. 5:

Support for TCEQ's Selection of Intestinal Effects as the Basis for the RfD

In addition to the intestinal tumors in mice, exposure to Cr(VI) was shown to increase oral cavity tumors in rats (NTP, 2008). Since TCEQ did not address these tumors specifically in the Development Support Document, we assume that TCEQ concluded that these tumors, which were only significantly elevated at 180 ppm Cr(VI), are not relevant for setting an oral RfD. We agree with this conclusion for the following reasons:

1. Questionable human relevance. Oral tumors were only significantly elevated (14% in males; 22% in females) in the highest treatment group, *viz*. 516 mg/L SDD ≈ 180 ppm Cr(VI) (NTP, 2008). With this highest dose group included, the Cochran Armitage Trend Test in U.S. EPA's BMD software is statistically significant; however, the trend test is not significant when the highest dose group is removed. This indicates that there is no statistical evidence of a dose-response at lower concentrations (OECD, 2006). The NOAEL from this study was 60 ppm Cr(VI), which greatly exceeds the average Cr(VI) concentration in U.S. drinking water (~0.001 ppm). This calls into question the human relevance of these data.

2. High margin of exposure (MOE). The margin of exposure (MOE) is defined as a $BMDL_{10}$ for tumors in an animal study divided by estimates of human exposure. The $BMDL_{10}$ for oral cavity tumors in rats (using data for females reported in U.S. EPA, 2010) is 3.5 mg/kg-day. Based on available water data (U.S. EPA, 2014), the mean Cr(VI) levels in U.S. drinking water are ~0.001 ppm; thus an 80 kg adult consuming 2.5L of water per day would be exposed to 3.1E-5 mg Cr(VI)/kg-day. Therefore, the MOE would be greater than 100,000. The geometric mean Cr(VI) concentration reported by another group is ~0.2 ppb (0.0002 ppm) (EWG, 2011), and thus the MOE would be over 500,000. MOE estimates based on other exposure statistics (e.g. 95th percentile) would be lower, but still in line with MOE values that indicate low concern for human health (Barlow *et al.*, 2006; COC, 2012).

3. No evidence of direct chemical action. No non-neoplastic or pre-neoplastic lesions have been observed in the oral cavity of rats or mice exposed to Cr(VI) (NTP, 2007; NTP, 2008;

Thompson *et al.*, 2011; Thompson *et al.*, 2012). This argues against direct chemically mediated effects.

4. No evidence of mutagenicity. Exposure to 180 ppm Cr(VI) did not increase mutant frequency (MF) in an *in vivo* transgenic mutation assay in Big Blue TgF344 rats exposed to 180 ppm Cr(VI) (Thompson *et al.*, 2015). In contrast, exposure to the mutagenic oral carcinogen 4-nitroquinoline-1-oxide significantly increased the MF in two regions of the oral mucosa (Thompson *et al.*, 2015).

5. No transcriptional (mRNA) response in oral mucosa. Exposure to Cr(VI) for 90 days elicits transcriptomic (mRNA) expression level changes in the duodenum of rats and mice in exposure groups where the tissue chromium concentration is greater than 10 mg/kg tissue (Kopec *et al.*, 2012a; Kopec *et al.*, 2012b). In the oral mucosa, however, tissue chromium levels never exceeded 10 mg/kg in rats or mice even after exposure to 180 ppm Cr(VI) for 90 days (Kirman *et al.*, 2012).

Consistent with the above findings, exposure to Cr(VI) for 90 days did not induce any significant ±2-fold changes in transcript levels in the rat oral mucosa (manuscript submitted for publication). Moreover, no significant ±2-fold changes in transcript levels occurred after 7 days of exposure. In mice, less than 20 genes were significantly altered after 7 days of exposure and only one gene after 90 days of exposure. These data further support #3 (above).

6. Evidence for poor health. Male mice in the NTP 90-day bioassay did not tolerate 180 ppm Cr(VI) (NTP, 2007) and thus received only up to 90 ppm Cr(VI) in the NTP (2008) 2-year cancer bioassay. The 2-fold higher 180 ppm dose in rats and female mice was likely toxic to these rodents. Rats in the NTP (2008) cancer bioassay exhibited dose-dependent decreases in water intake, bodyweight, and bodyweight gain. Decreased water intake can potentially affect saliva output, which might influence oral health. In the Cr(VI) MOA Research 90-day rat study, tissue and serum iron levels were significantly decreased, and transcriptomic responses in the duodenum were consistent with iron deficiency (Suh *et al.*, 2014). These data further indicate the potential for poor health in all animals chronically exposed to 180 ppm Cr(VI), which confounds the oral tumor data.

7. Inconsistent with mutagenic carcinogens. Generally speaking, mutagenic carcinogens often induce tumors in more than one tissue, and tend to induce tumors early in studies (U.S. EPA, 2007). Rats and mice in the NTP (2008) cancer bioassay only exhibited treatment-related tumors in one tissue: oral cavity of rats and small intestine of mice. Tumors in both locations occurred later in the study (after 500 days for oral tumors). Thus, the tumor profile for Cr(VI) is not consistent with a mutagenic MOA, which is supported by findings in #4 (above), and further calls into question the human relevance of the oral tumors in rats. 8. The proposed RfD is protective of oral tumors. Given all of the above, there is no evidence that environmentally relevant exposures of Cr(VI) increase oral cancer risk in rats, mice, or humans. The RfD proposed by TCEQ leads to drinking water standards of ~0.1 ppm, which is well below the concentrations that led to oral tumors in rats.

In summary, there are many lines of evidence that call into question the human relevance of the oral tumors in the NTP (2008) 2-year cancer bioassay. Moreover, the weight of the evidence suggest that the tumors were the result of indirect mechanisms not directly related to chromium tissue dosimetry.

TCEQ Response:

The TCEQ acknowledges that the RfD is protective of potential carcinogenic effects.

ACC Comments

Comment No. 1:

In June 2016, The Texas Commission on Environmental Quality (TCEQ) issued its Development Support Document (DSD) for hexavalent chromium [Cr(VI)] and proposed an oral reference dose (RfD) of 0.0031 mg/kg-day as protective against both potential non-cancerous and cancerous effects of Cr(VI) in drinking water. The method used to derive TCEQ's draft RfD appears consistent with the TCEQ guidelines (TCEQ 2015) and is based on a thorough assessment of the Cr(VI) database.

TCEQ Response:

The TCEQ acknowledges the robust, scientific defensibility and thoroughness of the assessment (e.g., methods, evaluation of MOA data).

Comment No. 2:

The US Environmental Protection Agency (EPA) undertook a three-year monitoring program for total chromium and Cr(VI) under its third Unregulated Contaminant Monitoring Rule and identified low levels of Cr(VI) in ground waters throughout the US that are used as sources for drinking water, including those in Texas (U.S. EPA 2014). It is imperative that any risk evaluation include data closely related to environmental exposures as part of its evaluation. Importantly, the TCEQ evaluation included assessing available data at the low end of the experimental range (100 ppb) to approximate environmental levels of Cr(VI) in drinking water. ACC supports the full use of the scientific database when regulatory agencies, such as TCEQ, undertake the development of a health value.

TCEQ Response:

The TCEQ acknowledges the thoroughness of the assessment in regard to the scientific database.

Comment No. 3:

In the DSD and the three peer reviewed published manuscripts (Haney 2015a, b, c), TCEQ outlined its critical evaluation of the evidence from the National Toxicology Program (NTP) and more recent Cr(VI) mode of action (MOA) studies published subsequent to the NTP study (reviewed in Thompson et al. 2013). TCEQ's derivation of the proposed RfD relies on 1) evidence for a non-

mutagenic threshold-based MOA for intestinal tumors observed in mice, 2) target tissue pharmacokinetic data, and 3) the application of conservative uncertainty factors.

ACC supports TCEQ's use of the entire, now enlarged, database of findings from the NTP studies (NTP 2007, 2008), the recent Cr(VI) MOA studies, and the other published data on Cr(VI). In particular, the Cr(VI) MOA data, whose animal dosing and experimental design were conducted to replicate as closely as possible the conditions of NTP animal studies, extends the NTP data considerably. The new data provides risk assessors with one of the most robust databases available, extending from high experimental doses to environmentally relevant levels, from which to evaluate the MOA of Cr(VI), as inputs into the derivation of risk values.

TCEQ Response:

The TCEQ acknowledges the thoroughness of the assessment in regard to the scientific database. Failure of a chemical assessment's low-dose extrapolation to appropriately evaluate the database and weight of evidence and incorporate (if scientifically robust and defensible) relevant data on the MOA and toxicokinetics may result in significantly overestimating environmental risk.

Comment No. 4:

The TCEQ RfD (0.0031 mg/kg-day) and Health Canada's proposed Tolerable Daily Intake (0.0044 mg/kg-day) (Health Canada 2015) values are very similar despite the differences in some of the methodologies used. We also note the similarities with the drinking water equivalent level (0.006 mg/kg-day) derived by Thompson et al. (2014). ACC concludes that these relatively similar values derived using varying methods should provide the regulatory community and public with the confidence that these levels are scientifically-based and protective against both potential non-cancerous and cancerous effects of Cr(VI) in drinking water.

TCEQ Response:

The TCEQ acknowledges the comment and further notes that: (1) Health Canada (2015) concurs that confidence in a cytotoxic MOA is high; and (2) use of the most conservative human equivalent dose (0.028 mg/kg-day) in recent PBPK evaluations by USEPA (Sasso and Schlosser 2015) as the point of departure would result in an identical RfD (rounded to one significant figure).

Comment No. 5:

ACC encourages TCEQ to adopt its proposed oral reference dose for hexavalent chromium.

TCEQ Response:

The proposed RfD has been adopted.