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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

J. Haney Jr.

Texas Commission on Environmental Quality (TCEQ), Austin, TX, United States

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ABSTRACT

The mouse dose at the lowest water concentration used in the National Toxicology Program hexavalent chromium (CrVI) drinking water study (NTP, 2008) is about 74,500 times higher than the approximate human dose corresponding to the 35-city geometric mean reported in EWG (2010) and over 1000 times higher than that based on the highest reported tap water concentration. With experimental and environmental doses differing greatly, it is a regulatory challenge to extrapolate high-dose results to environmental doses orders of magnitude lower in a meaningful and toxicologically predictive manner. This seems particularly true for the low-dose extrapolation of results for oral CrVI-induced carcinogenesis since dose-dependent differences in the dose fraction absorbed by mouse target tissues are apparent (Kirman et al., 2012). These data can be used for a straightforward adjustment of the USEPA (2010) draft oral slope factor (SFo) to be more predictive of risk at environmentally-relevant doses. More specifically, the evaluation of observed and modeled differences in the fraction of dose absorbed by target tissues at the point-of-departure for the draft SFo calculation versus lower doses suggests that the draft SFo be divided by a dose-specific adjustment factor of at least an order of magnitude to be less over-predictive of risk at more environmentally-relevant doses.

1. Introduction

In recent years, there has been a great deal of scientific debate and new research regarding exactly how and under what conditions CrVI is likely to induce cancer following oral exposure (e.g., Thompson et al., 2011a; McCarroll et al., 2010; USEPA, 2010). Some significant topics of debate concern issues relevant to the mode of action (MOA) and whether the excess risk observed at very high mouse oral doses of CrVI would be expected to extrapolate downward to significantly lower, truly environmentally-relevant human doses in a linear manner or if a nonlinear/threshold dose–response should be expected at such low doses. Such topics include the roles of mutagenicity and chronic hyperplasia in CrVI-induced carcinogenicity in target tissues, if the MOA and/or gastrointestinal (GI) extracellular reductive capacity likely impart a nonlinear/threshold character to the dose–response, and the potential that mouse oral doses in NTP (2008) exceeded the extracellular CrVI reductive capacity of the stomach/GI tract.

As part of the CrVI MOA research project (e.g., Thompson et al., 2011a), Proctor et al. (2012) report that stomach reducing capacity was likely exceeded at doses causing cancer in the mouse small intestine, and indicate that physiologically-based toxicokinetic (PBTK) models are necessary to account for competing kinetic rates in extrapolating target tissue dose for the purpose of risk assessment. If extracellular CrVI reductive capacity is exceeded at high drinking water concentrations such as those inducing cancer of the small intestine in NTP (2008), increased tissue uptake would be anticipated compared to lower doses (Thompson et al., 2011b). In other words, dose-dependent changes in the fraction of dose absorbed would be expected at doses which exceed stomach/GI extracellular CrVI reductive capacity compared to those that do not, with a higher dose fraction absorbed at doses exceeding reductive capacity.

In this study, tissue concentration data collected at various doses as part of the CrVI MOA research project (including some doses lower than those used in NTP, 2008) are evaluated to:

(1) quantify differences in the dose fraction absorbed at relevant doses; and
(2) derive factors based on dose-dependent changes in target tissue absorption that may be used to adjust the draft oral slope factor (SFo) to be more predictive of risk at lower, more environmentally-relevant doses.

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Table 1
Total chromium target tissue concentrations in B6C3F1 mice.[a]

Drinking water concentration (mg SDD/L)	Dose (mg Cr/kg-day)	Body weight[b] (g)	Total daily dose[c] (mg Cr/day)	Duodenum tissue concentration (mean mg Cr/kg tissue)	±SD	95% UCL[d] (mg Cr/kg tissue)	95% LCL[e] (mg Cr/kg tissue)	Jejunum tissue concentration (mean mg Cr/kg tissue)	±SD	95% UCL (mg Cr/kg tissue)	95% LCL (mg Cr/kg tissue)	Ileum tissue concentration (mean mg Cr/kg tissue)	±SD	95% UCL (mg Cr/kg tissue)	95% LCL (mg Cr/kg tissue)
0	0	25.8	0	0.017	0.007	0.022	0.012	0.046	0.044	0.078	0.014	0.020	0.01	0.027	0.013
0.3[f]	0.024	26.4	0.001	0.056	0.015	0.067	0.045	0.034	0.021	0.049	0.019	0.014	0.000	0.014	0.014
4	0.32	25.9	0.008	1.5	0.27	1.7	1.3	0.11	0.052	0.15	0.07	0.042	0.03	0.066	0.018
14	1.1	26.3	0.029	***7.3***	0.78	7.9	6.7	0.33	0.29	0.54	0.12	0.13	0.03	0.15	0.11
60	4.6	25.3	0.116	***33.5***	5.0	37.2	29.8	***4.7***	3.3	7.1	2.3	***0.92***	1.0	1.66	0.18
170	11.6	24.9	0.289	***42.4***	12.4	51.5	33.3	***21.6***	14.8	32.5	10.7	***1.8***	1.1	2.6	1.0
520	30.9	23.3	0.720	***60.9***	14.1	71.3	50.5	***13.9***	6.9	19.0	8.8	***2.3***	0.86	2.9	1.7

[a] Drinking water and tissue data taken from Table 3 of Kirman et al. (2012), who reported ***bold italicized*** values as significantly different than controls ($p < 0.05$).
[b] Body weight data from Table S2 of Thompson et al. (2011b).
[c] Calculated as mg Cr/kg-day × body weight in kilograms.
[d] 95%UCL = mean + (1.645 × SE) where SE = SD/n^0.5 and $n = 5$.
[e] 95%LCL = mean − (1.645 × SE) where SE = SD/n^0.5 and $n = 5$.
[f] Corresponds to the federal MCL of 0.1 mg Cr/L; MW of Cr_2/MW of SDD ≈ 104/298 ≈ 0.35 as conversion factor to convert SDD concentrations to Cr.

Table 2
Added chromium target tissue concentrations in B6C3F1 mice.[a]

Drinking water dose (mg Cr/kg-day)	Body weight[b](g)	Total daily dose[c] (mg Cr/day)	Duodenum tissue concentration (mean added mg Cr/kg tissue)	±SD	95% UCL[d] (added mg Cr/kg tissue)	95% LCL[e] (added mg Cr/kg tissue)	Jejunum tissue concentration (mean added mg Cr/kg tissue)	±SD	95% UCL (added mg Cr/kg tissue)	95% LCL (added mg Cr/kg tissue)	Ileum tissue concentration (mean added mg Cr/kg tissue)	±SD	95% UCL (added mg Cr/kg tissue)	95% LCL (added mg Cr/kg tissue)
0.024	26.4	0.001	0.039	0.015	0.050	0.028	0	0.021	0	0	0	0.000	0	0
0.32	25.9	0.008	1.5	0.3	1.7	1.3	0.068	0.052	5.78E−05	1.62E−05	0.021	0.033	0.045	−0.003
1.1	26.3	0.029	7.2	0.8	7.8	6.6	0.28	0.29	2.72E−04	3.68E−05	0.11	0.03	0.13	0.09
4.6	25.3	0.116	33.5	5.0	37.2	29.8	4.7	3.3	3.79E−03	1.21E−03	0.9	1.0	1.6	0.16
11.6	24.9	0.289	42.4	12.4	51.5	33.3	21.5	14.8	1.69E−02	5.55E−03	1.8	1.1	2.6	1.0
30.9	23.3	0.720	60.9	14.1	71.3	50.5	13.8	6.9	9.24E−03	4.27E−03	2.3	0.9	3.0	1.6

[a] Drinking water doses and added Cr (over background) tissue data taken from Table 8 of Kirman et al. (2012) with background shown as zero added.
[b] Body weight data from Table S2 of Thompson et al. (2011b).
[c] Calculated as mg Cr/kg-day × body weight in kilograms.
[d] 95%UCL = mean + (1.645 × SE) where SE = SD/n^0.5 and $n = 5$.
[e] 95%LCL = mean − (1.645 × SE) where SE = SD/n^0.5 and $n = 5$.

Table 3
Absorbed dose fraction estimates for the mouse duodenum.

Drinking Water Concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total Daily Dose (mg Cr/day)	Duodenum Fraction of Body Weight[b]	Duodenum Weight[c] (kg)	Duodenum Total Cr[d] (mg)	95% UCL Duodenum Total Cr (mg)	95% LCL Duodenum Total Cr (mg)	Mean Dose Fraction Absorbed[e]	95% UCL Dose Fraction Absorbed	95% LCL Dose Fraction Absorbed
0	0	0	0.012	3.10E-04	5.26E-06	6.86E-06	3.67E-06			
0.3[f]	0.024	0.001	0.012	3.17E-04	***1.77E-05***	2.12E-05	1.42E-05	1.97E-02	2.27E-02	1.67E-02
4	0.32	0.008	0.012	3.11E-04	4.66E-04	5.28E-04	4.04E-04	5.56E-02	6.29E-02	4.84E-02
14	***1.1***[g]	0.029	0.012	3.16E-04	***2.30E-03***	2.48E-03	2.12E-03	7.95E-02	8.57E-02	7.32E-02
60	4.6	0.116	0.012	3.04E-04	1.02E-02	1.13E-02	9.05E-03	8.73E-02	9.69E-02	7.78E-02
170	11.6	0.289	0.012	2.99E-04	1.27E-02	1.54E-02	9.94E-03	4.38E-02	5.33E-02	3.44E-02
520	30.9	0.720	0.012	2.80E-04	1.70E-02	1.99E-02	1.41E-02	2.36E-02	2.77E-02	1.96E-02

[a] Doses and total daily doses from Table 1.
[b] Tissue-specific fractions of body weight from Table 4 of Kirman et al. (2012).
[c] Calculated as fraction of body weight × body weight from Table 1.
[d] Calculated as tissue weight × tissue concentration (mean, 95% UCL, or 95% LCL) from Table 1; tissue concentrations associated with ***bold italicized*** values were compared to each other and are statistically significantly different by unpaired *t*-test ($p < 0.001$).
[e] Corrected for background concentrations in controls at 0 dose.
[f] Corresponds to the federal MCL of 0.1 mg Cr/L.
[g] Corresponds to the POD used for the draft SFo ($BMDL_{10}$ values of 1.0–1.2 mg/kg-day).

Table 4
Absorbed dose fraction estimates for the mouse jejunum.

Drinking Water Concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total Daily Dose (mg Cr/day)	Jejunum Fraction of Body Weight[b]	Jejunum Weight[c] (kg)	Jejunum Total Cr[d] (mg)	95% UCL Jejunum Total Cr (mg)	95% LCL Jejunum Total Cr (mg)	Mean Dose Fraction Absorbed[e]	95% UCL Dose Fraction Absorbed	95% LCL Dose Fraction Absorbed
0	0	0	0.021	5.42E-04	2.49E-05	4.25E-05	7.39E-06			
0.3[f]	0.024	0.001	0.021	5.54E-04	***1.88E-05***	2.74E-05	1.03E-05	0	0	4.58E-03
4	0.32	0.008	0.021	5.44E-04	5.98E-05	8.06E-05	3.90E-05	4.21E-03	4.61E-03	3.82E-03
14	***1.1***[g]	0.029	0.021	5.52E-04	***1.82E-04***	3.00E-04	6.44E-05	5.44E-03	8.91E-03	1.97E-03
60	4.6	0.116	0.021	5.31E-04	2.50E-03	3.79E-03	1.21E-03	2.12E-02	3.22E-02	1.03E-02
170	11.6	0.289	0.021	5.23E-04	1.13E-02	1.70E-02	5.60E-03	3.90E-02	5.87E-02	1.94E-02
520	30.9	0.720	0.021	4.89E-04	6.80E-03	9.29E-03	4.32E-03	9.41E-03	1.28E-02	5.99E-03

[a] Doses and total daily doses from Table 1.
[b] Tissue-specific fractions of body weight from Table 4 of Kirman et al. (2012).
[c] Calculated as fraction of body weight × body weight from Table 1.
[d] Calculated as tissue weight × tissue concentration (mean, 95% UCL, or 95% LCL) from Table 1; tissue concentrations associated with ***bold italicized*** values were compared to each other and practically achieved a statistically significant difference by unpaired *t*-test ($p = 0.052$).
[e] Corrected for background concentrations in controls at 0 dose, negative corrected values set to zero.
[f] Corresponds to the federal MCL of 0.1 mg Cr/L.
[g] Corresponds to the POD used for the draft SFo ($BMDL_{10}$ values of 1.0–1.2 mg/kg-day).

Table 5
Absorbed dose fraction estimates for the mouse ileum.

Drinking Water Concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total Daily Dose (mg Cr/day)	Ileum Fraction of Body Weight[b]	Ileum Weight[c] (kg)	Ileum Total Cr[d] (mg)	95% UCL Ileum Total Cr (mg)	95% LCL Ileum Total Cr (mg)	Mean Dose Fraction Absorbed[e]	95% UCL Dose Fraction Absorbed	95% LCL Dose Fraction Absorbed
0	0	0	0.0063	1.63E-04	3.25E-06	4.33E-06	2.17E-06			
0.3[f]	0.024	0.001	0.0063	1.66E-04	***2.33E-06***	2.33E-06	2.33E-06	0	0	2.43E-04
4	0.32	0.008	0.0063	1.63E-04	6.85E-06	1.08E-05	2.89E-06	4.35E-04	7.83E-04	8.65E-05
14	***1.1***[g]	0.029	0.0063	1.66E-04	***2.15E-05***	2.48E-05	1.82E-05	6.32E-04	7.09E-04	5.56E-04
60	4.6	0.116	0.0063	1.59E-04	1.47E-04	2.64E-04	2.94E-05	1.23E-03	2.23E-03	2.34E-04
170	11.6	0.289	0.0063	1.57E-04	2.82E-04	4.09E-04	1.55E-04	9.66E-04	1.40E-03	5.31E-04
520	30.9	0.720	0.0063	1.47E-04	3.38E-04	4.30E-04	2.45E-04	4.64E-04	5.92E-04	3.37E-04

[a] Doses and total daily doses from Table 1.
[b] Tissue-specific fractions of body weight from Table 4 of Kirman et al. (2012).
[c] Calculated as fraction of body weight × body weight from Table 1.
[d] Calculated as tissue weight × tissue concentration (mean, 95% UCL, or 95% LCL) from Table 1; tissue concentrations associated with ***bold italicized*** values were compared to each other and are statistically significantly different by unpaired *t*-test ($p < 0.001$).
[e] Corrected for background concentrations in controls at 0 dose, negative corrected values set to zero.
[f] Corresponds to the federal MCL of 0.1 mg Cr/L.
[g] Corresponds to the POD used for the draft SFo ($BMDL_{10}$ values of 1.0–1.2 mg/kg-day).

2. Materials and methods

Tissue concentration data reported by Kirman et al. (2012) were evaluated for this study. Kirman et al. report total and added chromium (Cr) mouse target tissue (i.e., duodenum, jejunum, ileum) concentrations that were collected to support the rodent PBTK model (Tables 1 and 2). In addition to drinking water concentrations used in the CrVI rodent drinking water study (NTP, 2008), these data include two lower water concentrations (0.3 and 4 mg sodium dichromate dehydrate (SDD)/L) and their corresponding daily Cr doses. The lowest water concentration tested for Kirman et al. corresponds to the federal maximum contaminant level for chromium (MCL of 0.1 mg Cr/L), making these data more relevant to possible environmental exposures than those from the NTP study (although still at concentrations and doses much higher than typical human exposures). Additionally, the current study uses USEPA benchmark dose (BMD) software (version 2.5) to model tissue concentration versus dose so that absorbed dose fractions corresponding to doses up to three times lower than the lowest tested for Kirman et al. (2012) can be calculated.

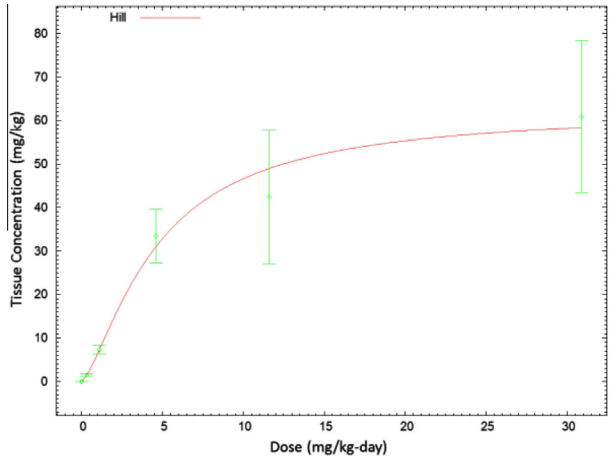

Fig. 1. Mouse duodenum tissue concentration versus daily dose.

Table 6
Duodenum best-fitting model tissue concentration prediction.

Hill Model (non-constant variance) equation:	Y [tissue conc. in mg/kg at dose] = intercept + v * dose^n/(k^n + dose^n)
Parameters	Inputs
Dose (mg/kg-day)[a]	0.008
Intercept	0.018
v	62.397
n	1.406
k	4.638
Solve for Y [tissue conc. in mg/kg at dose]	**0.026**

[a] Corresponds to one-third the mouse dose at the federal MCL.

The dose fractions absorbed into target tissues were calculated using the target tissue concentration data and tissue weights to first calculate the total amount of Cr in the target tissue (i.e., tissue concentration in mg Cr/kg tissue × tissue weight in kg = total mg Cr in tissue), and then dividing by the total daily dose (mg Cr/day, although use of cumulative dose would not change the relative differences in dose fraction absorbed at various doses). However, the target tissue concentration data presented in Table 1 for CrVI exposed mice are not corrected for the background Cr tissue levels present in control mice not exposed to CrVI. Thus, when calculating the CrVI dose fractions absorbed by these tissues (presented later in Tables 3–5), the background total Cr in a tissue was subtracted from that in exposed mice to represent only the additional Cr present in tissues due to the CrVI exposure (e.g., total Cr in a tissue due to CrVI exposure = total tissue Cr − background total tissue Cr in control mice). While this correction is not needed for the added Cr (over background) tissue concentration data presented in Table 2, data from both tables were used in order to evaluate and ensure consistency of results. The same process was used for 95% upper confidence limit (UCL) and 95% lower confidence limit (LCL) estimates. Accordingly, the dose fraction absorbed by a target tissue (i.e., duodenum, jejunum, ileum) at a given dose is calculated as follows:

$$\text{Dose Fraction Absorbed} = \text{Total Added Cr in Target Tissue} / \text{Cr Dose}$$

Absorbed dose fraction calculations based on the reported tissue concentration means, 95% UCL and 95% LCL estimates, and modeled tissue concentrations at even lower doses can be used to derive an adjustment factor for the draft SFo (0.5 per mg/kg-day; USEPA, 2010) to make it more predictive of excess risk at low doses, that is, doses lower than the point-of-departure (POD) used to calculate the SFo. More specifically, an evaluation of these tissue concentration data (based on both the empirical data collected and modeling the data) utilizing relatively straightforward calculations is used in this study to determine the factors by which the fractions of dose absorbed by target tissues decrease at lower, more environmentally-relevant doses compared to the POD made basis for the draft SFo ($BMDL_{10}$ values of 1–1.1 mg/kg-day). These factors account for dose-dependent changes in the dose fraction absorbed that are important to adjust for when the SFo is calculated based on a dose where an appreciably higher fraction is absorbed compared to the fractions absorbed at lower doses where the SFo will be used to estimate risk:

$$\text{Adjustment Factor} = DFA_{POD}/DFA_{ERD}$$

where:

DFA_{POD} = ∑ dose fractions absorbed by target tissues at the SFo POD; and DFA_{ERD} = ∑ dose fractions absorbed by target tissues at a lower, more environmentally-relevant dose where the SFo will be used to estimate risk.

Table 7
Absorbed dose fraction estimates based on modeled tissue concentrations for the mouse duodenum.

Drinking water concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total daily dose (mg Cr/day)	Duodenum tissue concentration[b] (mg Cr/kg tissue)	Duodenum total Cr[c] (mg)	Mean dose fraction absorbed[d]
1/3 the MCL	0.008	2.11E−04	0.026	8.19E−06	1.38E−02
1/2 the MCL	0.012	3.16E−04	0.032	1.02E−05	1.55E−02

[a] Doses and total daily doses at 1/3 and 1/2 the MCL were calculated based on these fractions × the doses at the MCL of 0.1 mg Cr/L (0.3 mg SDD/L) from Table 1.
[b] Tissue concentrations at 1/3 and 1/2 the MCL based on the BMD modeling equation in Table 6.
[c] Calculated as predicted tissue concentration x tissue weight at the MCL of 0.1 mg Cr/L (0.3 mg SDD/L) from Table 1.
[d] Corrected for the mean background duodenum tissue concentration in controls at 0 dose (0.017 mg Cr/kg tissue or total tissue Cr of 5.26E−06 mg) from Table 1.

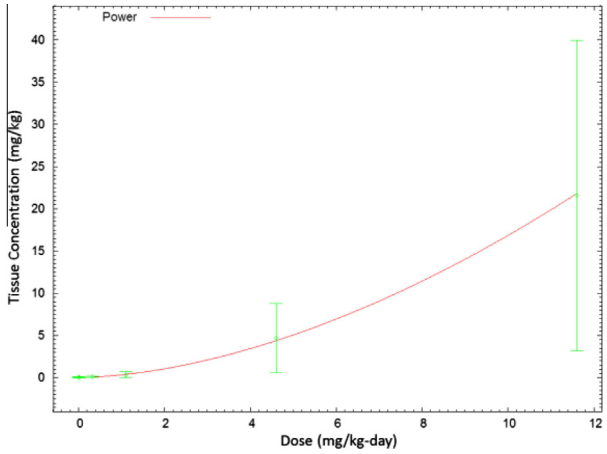

Fig. 2. Mouse jejunum tissue concentration versus daily dose.

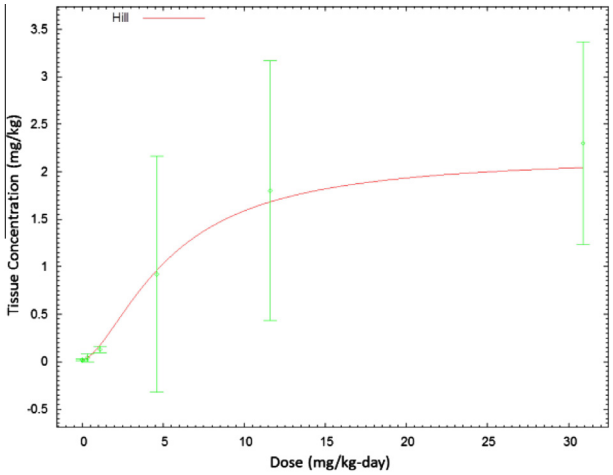

Fig. 3. Mouse ileum tissue concentration versus daily dose.

The draft SFo can simply be divided by this factor to be more predictive of risk a dose lower and more environmentally-relevant than those used in NTP (2008):

Adjusted SFo = SFo/Adjustment Factor

Lastly, examples of adjusted USEPA draft SFo values for CrVI are used to calculate excess risk at the federal MCL (0.1 mg/L) and a high but environmentally-relevant drinking water concentration (i.e., the maximum reported city drinking water concentration in EWG, 2010).

3. Results and discussion

There are dose-dependent differences in the dose fraction absorbed by target tissues (Tables 3–5) based on analysis of the tissue concentration data collected to support the rodent PBTK model (Kirman et al., 2012). As drinking water concentrations and associated doses increase from 0 to 60 mg SDD/L, the mean dose fractions absorbed also increase. This is true for all three target tissues, including the duodenum as the most carcinogenesis-responsive tissue (Table 3) and the jejunum (Table 4) as the secondary contributor to the draft SFo.

The dose fractions absorbed based on 95% UCL tissue concentrations also generally increase with dose as drinking water concentrations increase from 0 to 60 mg SDD/L. Based on 95% LCL tissue concentrations, the dose fractions absorbed by the duodenum (Table 3) also increase with dose over this drinking water concentration range (95% LCL results for the jejunum and ileum were more mixed).

While the dose fractions absorbed by target tissues increase with dose as drinking water concentrations increase from 0 to 60 mg SDD/L (Tables 3–5), it is apparent at the highest and least environmentally-relevant drinking water concentration doses (e.g., the two highest doses for the duodenum and ileum and the highest dose for the jejunum) that these tissues are unable to continue to absorb an ever-increasing fraction of the dose (although measured tissue concentrations are higher at these extremely high doses). Kirman et al. (2012) note that there is lower fractional absorption at the higher doses (>10 mg/kg-day at the two highest doses) where CrVI absorption is saturated, perhaps due to a toxic response (e.g., villi toxicity affecting transporter-mediated absorption and greater cell sloughing). However, as these higher doses are entirely irrelevant to environmental doses, this animal study high-dose phenomenon (CrVI absorption saturation at exceedingly high doses) does not detract from the significance of the results presented in the current paper for lower study doses that are still orders of magnitude higher than environmental exposures. For example, while the lowest water concentration tested in Kirman et al. of 0.3 mg SDD/L (0.1 mg Cr/L) is about 50 times less than the lowest concentration of 14.3 mg SDD/L tested in NTP (2008), it is still 555 times higher than the 35-city drinking water geometric mean (GM) and about 8 times higher than the city with the highest drinking water concentration (EWG, 2010).

In regard to more tissue- and dose-specific results, the duodenum and jejunum are the target tissues where the vast majority of adenomas/carcinomas were found in NTP (2008). Table 3 shows that the dose fraction absorbed by the mouse duodenum is approximately four times higher at the POD used for the draft SFo derivation than at the federal MCL (note that the duodenum tissue concentrations associated with these doses are statistically significantly different than each other; $p < 0.001$). This is significant given that the duodenum was the target tissue where most of

Table 8
Absorbed dose fraction estimates based on modeled tissue concentrations for the mouse jejunum and ileum.

Drinking water concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total daily dose (mg Cr/day)	Jejunum tissue concentration[b] (mg Cr/kg tissue)	Jejunum total Cr[c] (mg)	Mean dose fraction absorbed[d]	Ileum tissue concentration[b] (mg Cr/kg tissue)	Ileum total Cr[c] (mg)	Mean dose fraction absorbed[d]
1/3 the MCL	0.008	2.11E−04	0.0434	2.406E−05	0	0.0164	2.73E−06	0
1/2 the MCL	0.012	3.16E−04	0.0435	2.410E−05	0	0.0165	2.74E−06	0

[a] Doses and total daily doses at 1/3 and 1/2 the MCL were calculated based on these fractions × the doses at the MCL of 0.1 mg Cr/L (0.3 mg SDD/L) from Table 1.
[b] Jejunum and ileum tissue concentrations at 1/3 and 1/2 the MCL based on the BMD modeling equations (not shown) from best-fitting models.
[c] Calculated as predicted tissue concentration × tissue weight at the MCL of 0.1 mg Cr/L (0.3 mg SDD/L) from Table 1.
[d] BMD model-predicted jejunum and ileum tissue concentrations at 1/3 and 1/2 the MCL were just below the control (0 dose) background tissue levels of 0.046 and 0.020 mg Cr/kg tissue, respectively, so to correct for background tissue concentrations the dose fraction absorbed values at 1/3 and 1/2 the MCL for the jejunum and ileum were set to zero.

Table 9
Absorbed dose fraction estimates for the three mouse target tissues and SFo adjustment factors.

Drinking water concentration (mg SDD/L)	Dose[a] (mg Cr/kg-day)	Total daily dose (mg Cr/day)	Duodenum mean dose fraction absorbed[b]	Duodenum 95% UCL dose fraction absorbed	Duodenum 95% LCL dose fraction absorbed	Jejunum mean dose fraction absorbed[b]	Jejunum 95% UCL dose fraction absorbed	Jejunum 95% LCL dose fraction absorbed	Ileum mean dose fraction absorbed[b]	Ileum 95% UCL dose fraction absorbed	Ileum 95% LCL dose fraction absorbed	3-Tissue mean dose fraction absorbed[c]	3-Tissue 95% UCL dose fraction absorbed	3-Tissue 95% LCL dose fraction absorbed
1/3 MCL	0.008	2.11E−04	1.38E−02			0			0			**1.38E−02**		
1/2 MCL	0.012	3.16E−04	1.55E−02			0			0			1.55E−02		
0.3[d]	0.024	6.34E−04	1.97E−02	2.27E−02	1.67E−02	0	0	4.58E−03	0	0	2.43E−04	**1.97E−02**	**2.27E−02**	**2.15E−02**
4	0.32	8.29E−03	5.56E−02	6.29E−02	4.84E−02	4.21E−03	4.61E−03	3.82E−03	4.35E−04	7.83E−04	8.65E−05	6.03E−02	6.83E−02	5.23E−02
14	**1.1**[e]	2.89E−02	7.95E−02	8.57E−02	7.32E−02	5.44E−03	8.91E−03	1.97E−03	6.32E−04	7.09E−04	5.56E−04	**8.55E−02**	**9.53E−02**	**7.58E−02**
60	4.6	1.16E−01	8.73E−02	9.69E−02	7.78E−02	2.12E−02	3.22E−02	1.03E−02	1.23E−03	2.23E−03	2.34E−04	1.10E−01	1.31E−01	8.83E−02
170	11.6	2.89E−01	4.38E−02	5.33E−02	3.44E−02	3.90E−02	5.87E−02	1.94E−02	9.66E−04	1.40E−03	5.31E−04	8.38E−02	1.13E−01	5.43E−02
520	30.9	7.20E−01	2.36E−02	2.77E−02	1.96E−02	9.41E−03	1.28E−02	5.99E−03	4.64E−04	5.92E−04	3.37E−04	3.35E−02	4.11E−02	2.59E−02
									SFo adjustment factors[f]:					
									Based on lowest dose (at MCL)	**4.3**	4.2	3.5		
									Based on modeling at 1/3 MCL	**6.2**				

[a] Doses and total daily doses from Table 1, except doses at 1/3 and 1/2 the MCL were calculated based on these fractions × the doses at the MCL of 0.1 mg Cr/L (0.3 mg SDD/L).
[b] Mean and 95% UCL/LCL values from Tables 3–5 for doses tested in Kirman et al. (2012), and Tables 7 and 8 for mean dose fraction absorbed estimates at 1/3 and 1/2 the MCL.
[c] Sum of dose fractions absorbed (corrected for background tissue concentrations) for all three tissue mean, 95% UCL, or 95% LCL values.
[d] Corresponds to the federal MCL of 0.1 mg Cr/L.
[e] Corresponds to the POD used for the draft SFo ($BMDL_{10}$ values of 1.0–1.2 mg/kg-day).
[f] Calculated as dose fraction absorbed at draft SFo POD/fraction absorbed at the MCL or 1/3 the MCL.

the adenomas/carcinomas occurred in NTP (2008) and therefore was the principal contributor to the draft SFo. Similar to Table 3, Tables 4 and 5 show that the dose fractions absorbed into the mouse jejunum and ileum are higher at the POD used for the draft SFo derivation than at the federal MCL. This is of particular importance for the jejunum since this target tissue was a secondary contributor to the mouse adenomas/carcinomas observed in NTP (2008) and therefore contributed secondarily to the draft SFo. Not surprisingly, since the calculations in Tables 3–5 account for background tissue concentrations, essentially identical results were obtained using the added Cr tissue data provided in Table 2 (calculations not shown). Consequently, analyses based on data from Table 2 are not discussed further.

Greater differences in the dose fraction absorbed were found (compared to the dose fraction absorbed at the draft SFo POD) when duodenum tissue concentrations were modeled as a function of dose (BMD software version 2.5) in order to estimate absorbed dose fractions at one-half and one-third of the MCL. Fig. 1 shows good model fit (goodness-of-fit was evaluated by visual inspection with scaled residuals <|2| and a goodness-of-fit p value >0.1).

The equation and parameter estimates for this response function (provided by BMD software) were then used to calculate the estimated mean duodenum tissue concentrations at one-half and one-third of the MCL (3.2E−02 and 2.6E−02 mg/kg, respectively) since the MCL was the lowest water concentration for which data are provided in Kirman et al. (2012), drinking water concentrations are typically significantly below the MCL (e.g., EWG, 2010), and BMD modeling is generally not used to extrapolate to doses far below the experimental range (USEPA, 1995). For example, Table 6 provides the relevant inputs for the duodenum tissue concentration calculation at one-third of the federal MCL.

As in Table 3, these tissue concentrations and the total daily doses that would have been associated with one-half and one-third of the MCL (3.2E−04 and 2.1E−04 mg Cr/day, respectively) were used to estimate the dose fractions absorbed at these lower drinking water concentrations. The calculated absorbed dose fractions were approximately 1.6E−02 and 1.4E−02, respectively (Table 7).

Based on these results, the calculated dose fraction absorbed by the mouse duodenum is approximately six times higher at the POD used for the draft SFo derivation than at one-third of the federal MCL. This is significant given that most cancers occurred in this tissue in NTP (2008) and that typical drinking water concentrations are still almost 200 times lower than one-third the MCL (0.033 mg/L/35-city drinking water GM of 0.00018 mg/L ≈ 183).

Predictions by response function equations from good-fitting BMD models (Figs. 2 and 3) for the jejunum (high dose dropped) and ileum at even one-half the MCL (4.3E−02 and 1.6E−02 mg/kg, respectively) were slightly lower than mean background levels for those tissues (calculations not shown). Therefore, to account for background tissue concentrations, the dose fractions absorbed by these tissues (corrected for background) were set to zero at one-third and one-half of the MCL (Table 8).

Finally, in Table 9 absorbed doses by all three target tissues (from Tables 3–5, 7 and 8) are used to calculate overall SFo adjustment factors which account for differences in the dose fraction absorbed at the POD made basis for the draft SFo versus the fractions absorbed at lower, more environmentally-relevant doses (i.e., 1/3 the MCL and the lowest dose tested for Kirman et al., 2012).

The results in Table 9 show that the dose fraction absorbed by target tissues at the POD dose used in USEPA (2010) for the draft SFo calculation ($BMDL_{10}$ values of 1–1.1 mg/kg-day) is approximately four times higher than that at the MCL and about six times higher than that predicted at one-third of the MCL. Fig. 4 shows dose fraction absorbed versus dose for the lower drinking water concentrations of 0.3–60 mg SDD/L, which are closer to (although still significantly above) environmentally-relevant drinking water concentrations.

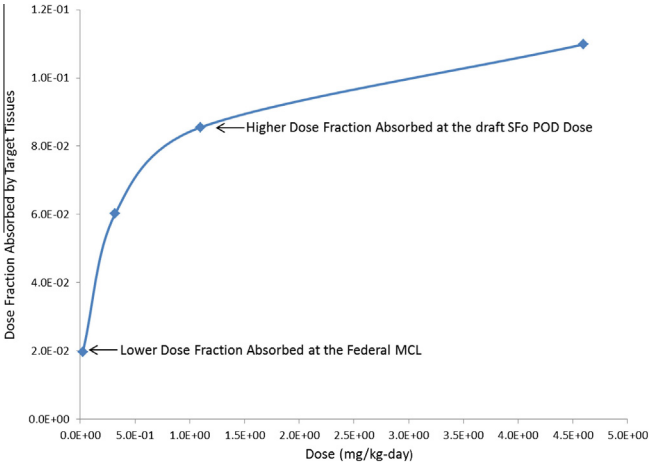

Fig. 4. Dose fraction absorbed versus dose.

Consideration of the shape of the curve in Fig. 4 suggests that using proportionality/linearity to estimate the expected dose fraction absorbed at truly low, environmentally-relevant water concentrations may be predictive. The 35-city drinking water GM (0.00018 mg/L) is over 500 times lower than the MCL (0.1 mg/L), the lowest water CrVI concentration tested in Kirman et al. (2012) and shown in Fig. 4. An estimate of the dose fraction absorbed at 0.00018 mg/L based on proportionality with the fraction absorbed at the lowest water concentration tested (0.3 mg SDD/L or 0.1 mg Cr/L) would be:

$$\text{Dose Fraction Absorbed}(\times)/0.00018\text{mgCr/L} = 1.97\text{E}-02/0.1\text{mgCr/L}$$

$$\text{Dose Fraction Absorbed}(\times) = (1.97\text{E}-02/0.1\text{mgCr/L}) \times 0.00018\text{mgCr/L}$$

$$\text{Dose Fraction Absorbed}(\times) = 3.55\text{E}-05$$

This estimate of the dose fraction which may be absorbed at the 35-city drinking water GM is over 2400 times lower than that calculated (8.55E−02 from Table 9) for the water concentration and dose (1.1 mg Cr/kg-day at 14 mg SDD/L) corresponding to the POD for the draft SFo (an adjustment factor of 2408). Even for the city with the highest drinking water concentration (0.0129 mg/L) reported in EWG (2010), the estimate of the dose fraction absorbed (2.54E−03; calculation not shown) is over 30 times lower than that calculated for the draft SFo POD (an adjustment factor of 34).

4. Conclusions

The above analyses show dose-dependent differences in the dose fraction absorbed by target tissues. More specifically, the dose fraction absorbed increases with dose from 0 to 60 mg SDD/L (0–21 mg Cr/L), which is up to 210 times the federal MCL (0.1 mg/L). Additionally, compared to the POD dose used in USEPA (2010) for the draft SFo calculation ($BMDL_{10}$ values of 1–1.1 mg/kg-day), analysis of the tissue concentration data collected (Table 9) indicates that the fractions of dose absorbed into target tissues of the mouse small intestine (duodenum, jejunum, ileum) are appreciably lower at lower doses. This may be due to dose-dependent changes

in the competing rates of reduction/detoxification prior to CrVI absorption by target tissues. Based on both the absorbed dose fractions calculated using measured target tissue concentration data and the absorbed fractions predicted at doses lower than those tested, it is further concluded that the magnitude of risk over-estimation by the draft SFo (0.5 per mg/kg-day) increases as it is used to estimate excess risk at progressively lower, more environmentally-relevant water concentrations where the dose fractions absorbed become progressively lower.

To be more predictive of risk, the draft SFo for CrVI should be adjusted by dose-specific adjustment factors (which vary) to account for the lower dose fractions absorbed by target tissues at lower, more environmentally-relevant water concentrations and doses as compared to the dose fraction absorbed at the water concentration and dose (1.1 mg Cr/kg-day at 14 mg SDD/L) corresponding to the POD for the draft SFo ($BMDL_{10}$ values of 1–1.1 mg/kg-day). For example, the 4-fold difference between the dose fraction absorbed at the MCL versus that at the water concentration corresponding to the draft SFo POD (see Table 9) indicates that the draft SFo over-predicts cancer risk by around four times even at the MCL (0.1 mg/L), (note that when the mouse dose at the MCL is converted to a human equivalent dose, the human dose is that expected for humans at the MCL) which is over 500 times higher than typical drinking water levels (e.g., 35-city GM of 0.00018 mg/L reported in EWG, 2010). Using this factor of 4 to adjust the draft SFo for the estimation of risk at the approximate human dose (2.9E−03 mg/kg-day) associated with the MCL results in an excess risk of about 3.6E−04. However, even the one-third of the MCL evaluated in this study is almost 200 times higher than typical drinking water concentrations (e.g., GM of 0.00018 mg/L), and the 6-fold difference between the dose fraction absorbed at one-third of the MCL versus that at the draft SFo POD (see Table 9) indicates that the draft SFo over-predicts cancer risk by around six times even at this high, atypical drinking water concentration (0.033 mg/L). In fact, the highest drinking water concentration (0.0129 mg/L) reported in EWG (2010) is only about one-eighth of the MCL. Perhaps even more pertinent to the propensity of the draft SFo to over-estimate environmental risk, estimates of the much lower dose fractions that may be absorbed at environmentally-relevant concentrations (0.00018–0.0129 mg/L) suggest that risk over-estimation by the draft SFo for drinking water concentrations that humans are likely to be exposed to may very well span orders of magnitude (tens to perhaps thousands). Furthermore, based on alternative MOAs, the carcinogenic risk at low (i.e., environmental) doses could be as low as zero (Thompson et al., 2013).

Considering that risk over-estimation by the draft SFo for environmentally-relevant drinking water concentrations is likely to be at least an order of magnitude and may span multiple orders of magnitude, these analyses suggest the draft SFo be divided by a dose-specific adjustment factor of at least an order of magnitude (adjusted SFo of $\leqslant$0.05 per mg/kg-day) to be less over-predictive of risk at human-relevant doses (e.g., 0.0129 mg/L). Use of an example adjusted SFo to conservatively estimate risk at the approximate human dose (3.7E−04 mg/kg-day) corresponding to the highest reported CrVI tap water concentration (0.0129 mg/L) from EWG (2010) results in a high-end excess risk estimate no greater than 1.9E−05. This CrVI drinking water risk is well within USEPA's acceptable risk range (1E−06–1E−04).

Potential limitations of this study include the assumption that 90-day tissue concentration data (Kirman et al., 2012) are representative of those for longer-term exposure (NTP, 2008) in a relative (not absolute) manner. That is, that the relative proportions of the dose fractions absorbed at various doses do not change significantly with longer exposure. The lack of target tissue data at truly environmentally-relevant drinking water concentrations and doses and the use of modeling (i.e., BMD, proportionality calculations) to account for this is another limitation and/or uncertainty associated with some analyses, although the information available supports the approaches utilized. Although the relative simplicity of the approach employed in the current study may be viewed as a limitation compared to the more elegant PBTK models that have been developed (Kirman et al., 2012, 2013), its straightforwardness and ease of understanding can also be viewed as strengths. An assumption inherent in adjusting an SFo in this manner based on dose fraction absorbed is that risk is proportional to target tissue dose. However, the assumption that target tissue dose is linearly related to risk is inherently part of the default linear low-dose extrapolation method commonly used in regulatory risk assessment and regarded as conservative (i.e., health protective, no threshold is assumed). On the other hand, although the present study assumes low-dose linearity of target tissue dose (not oral dose) and risk (i.e., a mutagenic MOA), this paper should not be viewed as an endorsement of it in the MOA debate, as this approach may not be the best supported low-dose extrapolation method for CrVI oral risk assessment (e.g., estimating risk at environmental doses) based on the available information relevant to the MOA for CrVI-induced oral carcinogenicity (e.g., Thompson et al., 2011a, 2013). Performing a weight-of-evidence on the most likely carcinogenic MOA, however, is beyond the scope of this paper.

Conflict of interest

Nothing to disclose.

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