Derivation of Ingestion-Based Soil Remediation Criteria for Cr^{+6} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate

Prepared by

Alan Stern, Dr.P.H., DABT
Division of Science, Research and Technology
New Jersey Department of Environmental Protection

for the

Risk Assessment Subgroup
of the
NJDEP Chromium Workgroup

April 8, 2009

Executive Summary

The National Toxicology Program (NTP) chronic bioassay of rats and mice exposed to sodium dichromate dihydrate in drinking water is the first study that provides data on the carcinogenicity of hexavalent chromium (Cr+6) by ingestion that is appropriate for quantitative risk assessment. Sodium dichromate dihydrate is readily soluble, yielding the dichromate ion that exists in equilibrium in solution with the chromate ion. The results of the NTP study are, therefore, applicable to the cancer risk assessment of Cr^{+6} by ingestion in general. NTP concluded that the study provides "clear evidence of carcinogenicity" in male and female mice and rats, based on benign and malignant tumors in rat oral mucosa and mouse small intestine. Consistent with the criteria for carcinogen characterization in the USEPA Guidelines for Carcinogen Risk Assessment, Cr^{+6} by ingestion is determined to be "likely to be carcinogenic to humans." The mouse was selected as the most sensitive species and the human cancer slope factor was developed based on assumptions and approaches that are consistent with the 2005 USEPA Guidelines for Carcinogen Risk Assessment. The human cancer slope factor was estimated to be 0.5 $(mg/kg/day)^{-1}$ based on the tumor incidence in male mice. Results from the combined data sets of male and female mice, while more uncertain, are consistent with these findings. Based on exposure assumptions for the oral exposure pathway in the NJDEP Soil Remediation Standards, this potency factor corresponds to a soil remediation criterion for Cr^{+6} of 1 ppm. Several lines of evidence support the conclusion that the observed carcinogenicity of Cr+6 did not result from exceedance of the inherent reduction capacity of the mouse gastrointestinal tract at the doses used in the NTP (2008) study. While the scientific literature provides ample data to support the conclusion that Cr^{+6} can act interact with DNA and can act as a mutagen, the NTP study provides evidence that additional modes of action (MOAs) may have functioned in the production of the mouse small intestine tumors.

Introduction

In July 2008, the National Toxicology Program (NTP) of the National Institutes of Health released its Final Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F1 Mice (NTP, 2008a) (http://ntp.niehs.nih.gov/files/546_web_FINAL.pdf). This report presents the results of a two-year chronic drinking water study of a highly soluble form of hexavalent chromium (Cr^{+6}). The draft final report was peer-reviewed by a panel of outside reviewers in May of 2007. The peer-review panel voted unanimously to accept the conclusions of "clear evidence of carcinogenicity" in male and female mice and rats. The final report carries these conclusions forward without substantive change.

Sodium dichromate dihydrate ($Na_2Cr_2O_7 \cdot 2H_2O$) is a common soluble compound of hexavalent chromium (Cr^{+6}). Previous studies on the health effects of the various forms of Cr^{+6}, including epidemiological studies of occupationally exposed cohorts have indicated that both common forms of hexavalent chromium, the chromate ion ($CrO_4^{=}$) and the dichromate ion ($Cr_2O_7^{=}$), are essentially identical in their toxicology. The only substantive difference is in their stoichiometry. That is, the dichromate ion contains two moles of Cr^{+6} for each mole of dichromate, whereas the chromate ion has only a single

mole of Cr^{+6} per mole of chromate. Therefore, the cancer potency and soil remediation values derived in this analysis and expressed in terms of the dose of Cr^{+6} apply equally well to both ions.

Brief Review of Previous Studies of the Carcinogenicity of Cr^{+6} by Ingestion

The following summary is not intended as a comprehensive review and discussion of the literature bearing on the carcinogenicity of Cr^{+6} by ingestion. It is presented to provide context for the interpretation of the NTP chronic bioassay data and its significance for the derivation of an estimate of the carcinogenicity of Cr^{+6} by ingestion.

The carcinogenicity of Cr^{+6} in the respiratory tract and particularly the lungs has been known since at least the 1930's from the experience of workers in the chromate industry. It is currently classified as a known human carcinogen by inhalation by the USEPA (2007a) and the International Agency for Research on Cancer (IARC) (2007). Despite some equivocal data that suggest an increased incidence of gastrointestinal tract cancers among chromate production workers, the earlier epidemiological literature did not provide a sound basis for assessing the carcinogenicity of Cr^{+6} by ingestion (reviewed in NJDEP, 2006). A recent re-analysis of population-based data on stomach cancer in China among residents in an industrial area whose drinking water was significantly contaminated by Cr^{+6} provides a stronger suggestion of the carcinogenicity of Cr^{+6} by ingestion (Beaumont et al., 2008). However, Beaumont et al. (2008) did not attempt to derive a quantitative dose-response relationship from their analysis and difficulties in quantifying exposure and directly linking exposure to cancer incidence make those data unsuitable for the development of a quantitative estimate of cancer potency.

Prior to the current NTP study, animal data on the carcinogenicity of hexavalent chromium by ingestion have been sparse. Borneff (1968) exposed three generations of mice to drinking water containing 500 ppm potassium chromate (K_2CrO_4). A statistically significant increase in stomach tumors was observed. However, this study is plagued by serious methodological problems, the most serious of which is that the mice experienced a high mortality due to a mouse pox epidemic during the course of exposure. The increase in tumors was seen almost exclusively in the generation most affected by the epidemic. This makes it likely that the observed increase in tumors was due, at least in part, to the infection. This observation makes this study unsuitable for assessment of oral carcinogenicity and/or for quantitative risk assessment. The Borneff et al. (1968) study is reviewed in greater detail in NJDEP Chromium Workgroup Report (NJDEP, 2006).

The only other study that directly addresses the oral carcinogenicity of Cr^{+6} is the study of Davidson et al. (2004) in which hairless female mice were supplied drinking water containing 0.1, 0.7, and 1.3 ppm Cr^{+6} as potassium chromate for 26 weeks and also exposed to UV light 2-3 times per week during this period. The UV light was in a range relevant to human exposure and was of sufficient wavelength and intensity to produce erythema. Comparison mice were exposed to only potassium chromate or to only UV light. Mice exposed to Cr^{+6} plus UV light developed significantly more skin tumors (benign plus malignant) than those exposed only to UV light, while mice with only Cr^{+6} exposure developed no skin tumors. These results were recently confirmed in male mice

(Uddin et al., 2007). This study provides strong evidence that Cr^{+6} can function as a co-carcinogen within the context of that study design. Of particular note in this study is the production of tumors at a site remote from the gastrointestinal tract despite the fact that the doses of Cr^{+6} in this study can be considered relatively low and potentially subject to reduction to the non-carcinogenic Cr^{+3} form within the gastrointestinal tract. This calls into question the previously posited theoretical ability of the gastrointestinal tract to completely reduce much larger doses of Cr^{+6} (Kerger et al., 1996a; De Flora et al., 1989; Petrilli and De Flora, 1988). Issues relating to reduction of Cr^{+6} are addressed in detail in Appendix A of this document. Nonetheless, use of this study as the basis for quantitative risk assessment is problematic because of its unusual design. The Davidson et al. (2004) study is reviewed in detail in the NJDEP Chromium Workgroup Report (NJDEP, 2006).

NTP Two-Year Ingestion Study Design

The NTP study exposed male and female F344/N rats and B6C3F1 mice to a constant concentration of Cr^{+6} in their sole source drinking water. Initially, there were 50 animals of each sex at each dose level. Concentrations of sodium dichromate were selected on the basis of an estimate of the maximum tolerated dose in earlier, subchronic (90 day) range finding study conducted by NTP (2007). Male and female rats were supplied with drinking water containing 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate for 2 years. Male mice were supplied with drinking water containing 0, 14.3, 28.6, 85.7, or 257.4 mg/L sodium dichromate dihydrate for 2 years. Female mice were supplied with 0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate for 2 years. The drinking water concentrations and their corresponding time-weighted doses as estimated by NTP are shown in Table 1.

Table 1.

Relationship among sodium dichromate dihydrate water concentration, sodium dichromate dihydrate dose and chromium dose in mice and rats

Rats

Males				Females			
sodium dichromate -dihydrate water conc. (mg/L)	Cr^{+6} water conc. (mg/L)	sodium dichromate-dihydrate dose (mg/kg/day)	Cr^{+6} dose [a] (mg/kg/day)	sodium dichromate -dihydrate water conc. (mg/L)	Cr^{+6} water conc. mg/L	sodium dichromate-dihydrate dose (mg/kg/day)	Cr^{+6} dose [a] (mg/kg/day)
0	0	0	0	0	0	0	0
14.3	5	0.6	0.21	14.3	5	0.7	0.25
57.3	20	2.2	0.77	57.3	20	2.7	0.95
172	60	6	2.1	172	60	7	2.45
516	180	17	5.95	516	180	20	7.00

Mice

Males				Females			
sodium dichromate water conc. (mg/L)	Cr^{+6} water conc. (mg/L)	sodium dichromate dose (mg/kg/day)	Cr^{+6} dose [a] (mg/kg/day)	sodium dichromate water conc. (mg/L)	Cr^{+6} water conc. mg/L	sodium dichromate dose (mg/kg/day)	Cr^{+6} dose [a] (mg/kg/day)
0	0	0	0	0	0	0	0
14.3	5	1.1	0.39	14.3	5	1.1	0.39
28.6	10	2.6	0.91	57.3	20	3.9	1.37
85.7	30	7	2.45	172	60	9	3.15
257.4	90	17	5.95	516	180	25	8.75

a. As reported by NTP.

Pathology and histopathology were performed on all major organ systems at approximately 730 days (~2 years) from the beginning of exposure (following sacrifice) or at the time of death for animals that died before the end of the study.

Brief Summary of Results

In both rats and male mice, a moderate decreased body weight was observed at the highest dose in surviving animals compared to controls. Based on the summary data presented in Table 11 of the NTP report, the time-weighted decreases at the highest doses averaged over the entire duration of the study were 7.5 and 8.0% for male and female rats, and 8.2 for male mice. For female mice, the time-weighted decrease in body weight at the highest dose was 20%. Table 2 summarizes the body weights over time during the course of the study for both sexes of rats and mice.

NTP stated that the relative decrease in body weight, was due in part, to decreased water consumption resulting from decreased palatability of the water. NTP further cited an analysis (unpublished) that examined the water intake of rats and mice in this study as a function of body weight. They state that through the first 20 weeks of dosing, male and

female rats and female mice drank approximately the same quantities of water per gram body weight as their respective controls. This was also the case for male mice except for those at the highest dose, which drank less water per gram body weight. In other words, high-dose male mice appeared to be restricting their normal water intake. Thus, it appears that palatability may only have been the primary cause of reduced water consumption for the high-dose male mice. Since water intake was not decreased in the female mice in the highest dose group, the decreased body weight in this group may, therefore, primarily reflect an intrinsic adverse effect of Cr^{+6} exposure. This suggests that at the highest dose, the female mice appear to have exceeded the maximum tolerated dose (MTD).
Table 2

Body Weight in Relation to Dose

RATS-M		**bw (g)**				
weeks	**fraction of total**	**controls**	**14.3 mg/L**	**57.3 mg/L**	**172 mg/L**	**516 mg/L**
1-13	0.13	261	257	257	252	243
14-52	0.39	457	449	453	441	427
53-101	0.49	523	514	518	502	477
weighted aver		468.43	460.38	463.9	450.73	431.85
% change from controls			-1.71851	-0.96706	-3.77858	-7.80906

RATS-F		**bw (g)**				
weeks	**fraction of total**	**controls**	**14.3 mg/L**	**57.3 mg/L**	**172 mg/L**	**516 mg/L**
1-13	0.13	163	160	160	158	157
14-52	0.39	245	238	237	233	228
53-101	0.49	326	316	318	311	294
weighted aver		276.48	268.46	269.05	263.8	253.39
% change from controls			-2.90075	-2.68736	-4.58623	-8.35142

MICE-M		**bw (g)**				
weeks	**fraction of total**	**controls**	**14.3 mg/L**	**28.6 mg/L**	**85.7 mg/L**	**257 mg/L**
1-13	0.13	32.8	33	33.3	32.1	29.4
14-52	0.39	51.6	51.6	51.8	51.1	46.7
53-101	0.49	53.8	53	52.3	52.7	49.8
weighted aver		50.75	50.384	50.158	49.925	46.437
% change from controls			-0.72118	-1.1665	-1.62562	-8.49852

MICE-F		**bw (g)**				
weeks	**fraction of total**	**controls**	**14.3 mg/L**	**57.3 mg/L**	**172 mg/L**	**516 mg/L**
1-13	0.13	24.6	24.4	23.7	23	22.1
14-52	0.39	50.1	49.9	47.2	42.9	37.2
53-101	0.49	61.9	62.4	60.2	57.1	50.7
weighted aver		53.068	53.209	50.987	47.7	42.224
% change from controls			0.265697	-3.92138	-10.1153	-20.4342

The NTP report specifically addresses the question of whether decreased water consumption resulted in dehydration. NTP noted that physical signs associated with

dehydration (loss of skin turgor, dry mucous membranes, retraction of eyes, hypoactivity, poor hair coats) were absent in both species. NTP also noted that hematologic parameters were measured in male rats at intervals during dosing through the first year of exposure. Parameters typically associated with dehydration (increases in hematocrit, serum albumin, total protein, urea nitrogen, and urine specific-gravity) were not observed. In contrast, significant *decreases* in hematocrit, serum albumin and total protein were noted in female mice, particularly at the two highest doses (hematologic analysis was not carried out on male mice). Based on these observations, NTP concluded that the observed increases in tumors could not be attributed to dehydration. Furthermore, we are unaware of any evidence from the scientific literature that suggests that dehydration can potentiate the development of tumors. No clinical signs of toxicity were observed in either species.

There was little difference in survival at termination at the highest dose compared to controls in either rats or mice, with a single animal (3%) comprising the maximum decrease. Clinical signs were normal at all doses. The only significant toxicity noted in either species was a statistically significant increase in neoplasms of the mucosa of the oral cavity and tongue in rats, and of the small intestine (duodenum, ileum, and jejunum) in mice. Combined neoplasms at these locations at the highest dose in male and female rats and at the two highest doses in male and female mice were statistically significantly elevated compared to controls.

The tumors of the oral mucosa seen in exposed rats have not previously been reported in the NTP database of historic drinking water controls. The combined tumors of the tongue and oral mucosa seen in exposed rats have a very low historic incidence among historic NTP drinking water controls (0.3 and1.2% for males and females respectively). The combined intestinal lesions seen in the exposed mice also occur with a low incidence in the NTP database of historic drinking water controls (3.7 and 1.1% for males and females respectively. NTP notes, however, that comparison to the concurrent (i.e., in-study) controls is the appropriate basis for statistical analysis of dose-response.

The incidence of neoplasms at the doses that were significantly elevated above the concurrent controls were also significantly elevated above the historic controls. In the rats, non-neoplastic lesions of the oral mucosa were not seen. This is consistent with the neoplastic lesions arising independently of necrotic tissue damage. In the mice, a low incidence of focal epithelial hyperplasia in the small intestine was noted. Its incidence was not dose related, but was, nonetheless, considered to be pre-neoplastic. Diffuse epithelial hyperplasia in the duodenum was significantly elevated in both sexes compared to controls at all doses and at the highest dose in the jejunum in female mice. The diffuse hyperplasia was characterized by several layers of cells piled up along the long axis of the intestinal villi. Intestinal crypts were elongated and contained increased number of cells with increased numbers of mitotic figures. NTP considers this diffuse hyperplasia to be consistent with regenerative cell growth secondary to tissue injury.

Selection of Key Species

Figures 1-4 show the incidence of oral neoplasms (rats) or neoplasms of the small intestine (mice). To avoid confusion, the tumor incidence in these figures is not adjusted for the number of animals at-risk. This is explained more fully below in the section, Calculation of Tumor Incidence and in Table 2. These adjustments do not affect the selection of the key species for calculation of cancer potency. The tumor incidence in both species demonstrates a dose-response. However, the response in the rats is mostly or entirely at the highest dose, whereas the response in the mice is observed at least in the two highest doses. In addition, the magnitude of the response in the mice at the highest dose is more than twice that in the rats. It is therefore clear that, in this study, the mouse is the more sensitive species. The mouse is, therefore, selected as the key species for derivation of the cancer potency by ingestion and the related soil remediation criterion.

General Approach for Calculating the Cancer Potency

The current USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a) state that: "When the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach." To date, no mode of action has been unambiguously demonstrated for Cr^{+6} carcinogenicity. Thus, under the current USEPA Guidelines, linear extrapolation is the appropriate approach for calculating Cr^{+6} oral cancer potency from these data. However, some scientific evidence suggests that tumors arise from the interaction of Cr^{+6} with DNA either directly, or through intra-cellular metabolism to Cr^{+3} (Kirpnick-Sobol et al., 2006; Dana Devi et al., 2001; Cohen et al., 1993; Coogan et al., 1991). These data suggest the possibility of a mutagenic mode of action, and , linear extrapolation is also the USEPA recommended approach when a mutagenic mode of action has been demonstrated.

With regard to linear extrapolation, the USEPA Guidelines also state that: "The linear approach is to draw a straight line between a point of departure from observed data, generally as a default, an LED [lower bound on the effective dose] chosen to be representative of the lower end of the observed range, and the origin (zero incremental dose, zero incremental response)." Consistent with the USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a), the point of departure (POD) is identified here as the lower confidence bound on the benchmark dose (BMDL). This concept is explained in detail below. The USEPA has used the BMDL as the point of departure in calculating oral cancer slope factors for several chemicals (e.g., 1,2-dibromomethane (USEPA, 2004); dichloroacetic acid (USEPA, 2003)). Also consistent with the USEPA Guidelines, the tumor incidence is based on the sum of benign and malignant tumors in the same tissue under the assumption that benign tumors have the potential to progress to malignancy when caused by an agent that also causes malignant tumors at the same site (USEPA, 2005a).

Determination of Cr^{+6} Dose

The exposure of the animals in the NTP bioassay was originally expressed in terms of the concentration of sodium dichromate dihydrate in their drinking water (mg/L). However,

cancer potency is expressed in terms of the inverse of *dose* (i.e., $(mg/kg\text{-}bw/day)^{-1}$). In addition, the results of the bioassay are used here to derive a generalized cancer potency estimate and an associated soil remediation criterion for Cr^{+6} rather than for sodium dichromate *per se*. The NTP provided an estimate of the dose of sodium dichromate dihydrate for each species and sex corresponding to each water concentration. The corresponding Cr^{+6} dose is obtained by multiplying the sodium dichromate dihydrate dose by 0.35, the fraction of the sodium dichromate dihydrate molecular weight contributed by chromium (see Table 1).

Body Weight

In order to calculate a human risk-specific dose from the animal cancer potency estimate, it is necessary to consider the animals' body weight. Since the cancer potency estimate derived from the animal data integrates dose-response data (including body-weight) across dose groups, a single representative value for animal body weight is required. The time-weighted average body weight for the control mice is selected. The time-weighted value is derived from the summary data reported in Table 11 of the NTP report. These values are 0.050 kg and 0.053 kg for male and female mice respectively.

The decrease in body-weight in male mice at the highest dose and second highest doses was 8.2% and 1.7% respectively and in the female mice was 20% and 10.2% respectively. Given these differences between the body weight of control and high-dose animals, the impact of the choice of the control mice to represent body weight for all dose groups can be seen in the following sensitivity analysis. For female mice (given the allometric dose scaling from mice to humans – see below), use of the time-weighted average body weight from control animals results in a human-specific cancer potency estimate that is approximately 6% larger than that calculated on the basis of the time-weighted average body weight at the highest dose (depending on the specific benchmark dose model employed – see below). The difference in the human cancer potency estimate when comparing the control body weight to the body weight for all other doses for females and to all doses for male mice would be less than 5%. Therefore, the derivation of cancer potency and risk-based guidance is not highly sensitive to the choice of body weight from among the various dose groups and the use of the time-weighted average control body weight is judged to be appropriate.

Calculation of Tumor Incidence

Dose-response analysis requires data for both dose and incidence. The ultimate goal in this analysis is the determination of the risk of the occurrence of at least one tumor occurring in a person as a result of exposure to a given dose of Cr^{+6}. Therefore, in this analysis, incidence is defined as the number of animals with at least one tumor divided by the number of animals at risk of developing a tumor. The numerator of this ratio is the sum of all mice in which a tumor (adenoma or carcinoma) was detected in at least one of the three sections of the small intestine – the duodenum, ileum, and jejunum. The denominator of this ratio, the number of animals at-risk of developing a tumor, includes all mice that survived long enough to have potentially experienced a tumor. Since the first tumor of the small intestine was recorded at day 451 of exposure, it is assumed that animals that died prior to that time were not at-risk.

NTP has provided both summary information on tumor incidence (Table 13 of the NTP final report) and individual animal pathology data (http://ntp.niehs.nih.gov/go/29141). The individual animal pathology data reflect microscopic examination of the small intestines whereas the summary data reflect both gross and microscopic examination. Not all segments of the small intestine were available for microscopic examination in all mice. However, all sections of the intestines of all mice were grossly examined by multiple examiners. Information supplied by NTP[1] indicates that nearly all tumors were identified at least by gross examination and that it is unlikely that any intestinal neoplasms were missed due to the unavailability of samples for microscopic examination. NTP therefore recommends that, in general, the denominator of the incidence should be the number of animals in each dose group (i.e., 50)[1]. We agree with this recommendation, with the exception of animals that died prior to day 451 of dosing. In Table 3, the number of animals at-risk is, therefore, equal to 50 minus the number of animals that died prior to day 451. The numerator of the incidence ratio is the total number of mice identified by NTP with small intestinal tumors and reflects the sum of tumors identified through gross and microscopic examination. That number is used as reported in Table 13 of the NTP final report.

Table ~~2~~.3

Estimated Tumor Incidence by Dose

	at risk	mice with neo-plasms	Inci-dence	at risk	mice with neo-plasms	Inci-dence	at risk	mice with neo-plasms	Inci-dence	at risk	mice with neo-plasms	Inci-dence	at risk	mice with neo-plasms	Inci-dence
	sodium dichromate water conc. (mg/L)														
mice M	**0**			**14.3**			**28.6**			**85.7**			**257.4**		
	49	1	0.020	49	3	0.061	49	2	0.041	50	7	0.140	48	20	0.417
mice F	**0**			**14.3**			**57.3**			**172**			**516**		
	49	1	0.020	50	1	0.020	49	4	0.082	49	17	0.347	49	22	0.449

Determination of the Point of Departure (POD)

As discussed above, the current approach under the USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a) for linear extrapolation of cancer dose-response considers that, in general, a POD is a lower effective dose (LED) chosen to be representative of the lower end of the observed range of response. It is not necessary that the LED be one of the administered doses. The benchmark dose approach was used to identify an appropriate POD. The USEPA's Integrated Risk Information System (IRIS)

[1] Dr. David Malarkey, NTP - personal communication 3/15/09

defines a benchmark dose as "a dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background (USEPA, 2007b)." For dichotomous data (e.g., tumor incidence), the BMR is generally chosen to represent the lowest level of response that is reasonably consistent with the observed data. For well designed bioassays, this value is often 0.1 or 0.05 (10% or 5% response) depending on the specific data. As can be seen in Figs. 5 and 6, a response rate of 0.1 (but not 0.05) falls within the ascending portion of the dose-response in both male and female mice but is close to the lowest dose showing a positive response. A BMR of 0.1 was, therefore, selected.

In applying the benchmark dose approach to the derivation of risk-based standards and guidance, the standard approach is to calculate a lower 95% confidence bound on the dose corresponding to the BMR (i.e., the benchmark dose or BMD). This lower confidence bound is referred to as the BMDL. For a BMR of 0.1, the corresponding BMDL is referred to as the $BMDL_{0.1}$. The use of the lower confidence bound on the benchmark dose is intended to account for uncertainty in the fit of the dose-response model to the data (see below).

Benchmark dose modeling was carried out using the USEPA's BMDS (version 1.4.1) software package (USEPA, 2007c). The BMDS software offers several possible mathematical dose-response functions for use with dichotomous data: logistic; gamma multi-hit; Weibull; quantal linear; probit; and multi-stage cancer. None of these dose-response functions has a biological basis that is necessarily specific to Cr^{+6} carcinogenicity. The fit of the data to each model is described in the BMDS software through the calculation of the chi-squared goodness-of-fit statistic and its corresponding p-value. As there is no biological basis for selecting any of the models, it could be argued that the model which best fits the data should be chosen. However, USEPA gives preference to the multi-stage cancer model because of its historic use as the USEPA's default for cancer dose-response modeling prior to POD-benchmark dose approach.

Because neoplasms of the small intestine occur spontaneously to some extent in the B6C3F1 strain of mouse, it is necessary to account for this background frequency in the dose modeling. This was addressed by modeling "extra risk." Extra risk is defined as the probability of the occurrence of the effect (i.e., a tumor) that can be specifically attributed to the dose for the animals at risk. Mathematically, this is expressed as $P_{D\text{-}ER} = (P_D - P_0)/(1 - P_0)$, where $P_{D\text{-}ER}$ is the probability of a tumor at dose D under extra risk; P_D is the observed probability of a tumor at dose D; and P_0 is the observed probability of a tumor at zero dose in the in-study controls (the background probability).

Benchmark dose modeling was carried out for the male and female mice separately (Tables 4a and 4b respectively), as well as for the combined male and female data sets (Table 4c). In addition, because the highest dose in the female rats appears to exceed the maximum tolerated dose (MTD), benchmark dose was also carried out for the combined male and female data sets with the high-dose females removed. This is referred to as the combined-reduced data set (Table 4d).

Results of POD Calculations

The first three columns of Tables 4a-d present the BMDL calculations that are used for the determination of the POD. For the male mice, all of the models had nearly identical fits to the data and the BMDL values all fell within a narrow range. In fact, with the exception of the probit model, all of the values were within 0.01 of each other. For the female mice, the BMDL values are more variable and none of the models gives a strong fit to the data as reflected by the low chi-square p-values. For the combined male and female mouse data (Table 4c), only the multi-stage cancer model gave a marginally acceptable fit. For the combined-reduced data set (Table 4d), the Weibull, gamma multi-hit, and quantal linear models each gave marginally acceptable fits. For each of the models that gave marginally acceptable fits to the data, the BMDL values were in good agreement with those from for the male mice. Each of the models gave a better fit to the male-only data than the fit of any of the models to any of the other data sets. Figures 5-8 show the fits of the best fitting model for each data set. The BMDL values obtained for the male data for each of the models are consistent with the BMDL values for each of the best fitting model in both of the combined data sets. Therefore, the derivation of the cancer potency estimate and corresponding soil cleanup value is based on the male mouse data.

.

Table 4a Calculation of cancer potency and soil conc. at 1 x 10^{-6} cancer risk – Male mice

mice M	2	3	4	5	6	7	8	9	10	11	12
BMD model	$BMDL_{0.1}$ mg/kg/d (extra risk)	chi-sq p-value	model response at BMDL	model response at 0 dose	slope from BMDL to 0 dose	animal dose at 1x10^{-6} risk mg/kg/d	time weighted av. study bw at 0 dose (kg)	human dose at 1x10^{-6} risk (mg/kg/d) based on $(bw)^{3/4}$ scaling and 70 kg bw	human cancer potency slope $(mg/kg/d)^{-1}$	mg Cr^{+6}/d at 1x10^{-6} risk for 59 kg av bw	soil conc. Cr^{+6} at 1x10^{-6} risk for 114 mg soil/d ppm
logistic	1.17	0.57	0.1	0	0.09	1.17E-05	0.05	1.91E-06	0.52	1.13E-04	0.99
weibulll	1.17	0.57	0.1	0	0.09	1.17E-05	0.05	1.91E-06	0.52	1.13E-04	0.99
probit	1.73	0.57	0.1	0	0.06	1.73E-05	0.05	2.83E-06	0.35	1.67E-04	1.46
gamma	1.17	0.56	0.1	0	0.09	1.17E-05	0.05	1.91E-06	0.52	1.13E-04	0.99
multi hit quantal linear	1.17	0.57	0.1	0	0.09	1.17E-05	0.05	1.91E-06	0.52	1.13E-04	0.99
multistage cancer	1.18	0.60	0.1	0	0.09	1.18E-05	0.05	1.93E-06	0.52	1.14E-04	1.00

Table 4b Calculation of cancer potency and soil conc. at 1 x 10^{-6} cancer risk – Female mice

mice F	2	3	4	5	6	7	8	9	10	11	12
BMD model	BMDL mg/kg/d (extra risk)	chi-sq p-value	model response at BMDL	model response at 0 dose	slope from BMDL to 0 dose	animal dose at 1x10-6 risk mg/kg/d	time weighted av. study bw at 0 dose (kg)	human dose at 1x10^{-6} risk (mg/kg/d) based on $(bw)^{3/4}$ scaling and 70 kg bw	human cancer potency slope $(mg/kg/d)^{-1}$	mg Cr+6/d at 1x10-6 risk for 59 kg av bw	soil conc. Cr+6 at 1x10-6 risk for 114 mg soil/d ppm
logisitic	2.64	0.00	0.1	0	0.04	2.64E-05	0.053	4.38E-06	0.23	2.58E-04	2.27
weibulll	0.67	0.06	0.1	0	0.15	6.70E-06	0.053	1.11E-06	0.90	6.56E-05	0.58
probit	2.44	0.00	0.1	0	0.04	2.44E-05	0.053	4.05E-06	0.25	2.39E-04	2.09
gamma	0.68	0.06	0.1	0	0.15	6.80E-06	0.053	1.13E-06	0.89	6.66E-05	0.58
multi hit quantal linear	0.67	0.06	0.1	0	0.15	6.70E-06	0.053	1.11E-06	0.90	6.56E-05	0.58
multistage cancer	1.03	0.13	0.1	0	0.10	1.03E-05	0.053	1.71E-06	0.59	1.01E-04	0.88

Table 4c Calculation of cancer potency and soil conc. at 1 x 10^{-6} cancer risk – Combined Male and Female Mice

mice F	2	3	4	5	6	7	8	9	10	11	12
BMD model	BMDL mg/kg/d (extra risk)	chi-sq p-value	model response at BMDL	model response at 0 dose	slope from BMDL to 0 dose (mg/kg/day)$^{-1}$	animal dose at 1x10-6 risk mg/kg/d	time weighted av. study bw at 0 dose kg	human dose at 1x10^{-6} risk (mg/kg/d) based on (bw)$^{3/4}$ scaling and 70 kg bw	human cancer potency slope (mg/kg/d)$^{-1}$	mg Cr+6/d at 1x10-6 risk for 59 kg av bw	soil conc. Cr+6 at 1x10-6 risk for 114 mg soil/d ppm
logisitic	2.67	0.00	0.1	0	0.04	2.67E-05	0.053	4.43E-06	0.23	2.61E-04	2.29
weibulll	1.00	0.20	0.1	0	0.10	1.00E-05	0.053	1.66E-06	0.60	9.79E-05	0.86
probit	2.47	0.00	0.1	0	0.04	2.47E-05	0.053	4.10E-06	0.24	2.42E-04	2.12
gamma	1.02	0.21	0.1	0	0.10	1.02E-05	0.053	1.69E-06	0.59	9.98E-05	0.88
multi hit quantal linear	1.00	0.20	0.1	0	0.10	1.00E-05	0.053	1.66E-06	0.60	9.79E-05	0.86
multistage cancer	1.12	0.3	0.1	0	0.09	1.12E-05	0.053	1.86E-06	0.54	1.10E-04	0.96

Table 4d Calculation of cancer potency and soil conc. at 1 x 10^{-6} cancer risk – Combined Male and Reduced Female Mice [a]

mice F	2	3	4	5	6	7	8	9	10	11	12
BMD model	BMDL mg/kg/d (extra risk)	chi-sq p-value	model response at BMDL	model response at 0 dose	slope from BMDL to 0 dose (mg/kg/day)$^{-1}$	animal dose at 1x10-6 risk mg/kg/d	time weighted av. study bw at 0 dose kg	human dose at 1x10^{-6} risk (mg/kg/d) based on (bw)$^{3/4}$ scaling and 70 kg bw	human cancer potency slope (mg/kg/d)$^{-1}$	mg Cr+6/d at 1x10-6 risk for 59 kg av bw	soil conc. Cr+6 at 1x10-6 risk for 114 mg soil/d ppm
logisitic	2.14	0.04	0.1	0	0.05	2.14E-05	0.053	3.55E-06	0.28	2.09E-04	1.84
weibulll	1.11	0.31	0.1	0	0.09	1.11E-05	0.053	1.84E-06	0.54	1.09E-04	0.95
probit	1.98	0.09	0.1	0	0.05	1.98E-05	0.053	3.28E-06	0.30	1.94E-04	1.70
gamma	1.13	0.32	0.1	0	0.09	1.13E-05	0.053	1.87E-06	0.53	1.11E-04	0.97
multi hit quantal linear	1.11	0.31	0.1	0	0.09	1.11E-05	0.053	1.84E-06	0.54	1.09E-04	0.95
multistage cancer	1.07	0.25	0.1	0	0.09	1.07E-05	0.053	1.77E-06	0.56	1.05E-04	0.92

a –The combined male and reduced female data set consists of the entire male mouse data set combined with the female mouse data set after removal of the high dose females

Results of Mouse Cancer Potency Slope Calculation

Column 6 of Table 4a-d gives the slope in $(mg/kg/day)^{-1}$ of the line between the point at zero dose-zero response and the point at $BMDL_{0.1}$-0.1 response. This is illustrated by the dashed line in Figure 5-8. This is the linear extrapolation approach described by the USEPA (2005a) cancer guidelines. The potency can also be expressed in terms of the dose predicted to result in one-in-a-million ($1x10^{-6}$) cancer risk to the mice in this study. This is given in column 7 of Table 4a-d.

Calculation of the Human Equivalent Dose

To convert the animal dose corresponding to $1x10^{-6}$ risk to the dose corresponding to the same risk in humans, the USEPA cancer guidelines recommend the allometric conversion on the basis of body weight to the ¾ power to address differences between species in metabolism and toxicokinetics related to body mass (USEPA, 2005a). When using this conversion to scale doses between animals and humans, the appropriate formula is HED = $(ABW/HBW)^{0.25}$ x AD where HED is the human equivalent dose (mg/kg/day), ABW is the animal body weight (kg), HBW is the human body weight (default value of 70 kg), and AD is the animal dose (mg/kg/day) (Rodricks et al., 2001). The straightforward calculation of the human equivalent dose requires the assumption of a single animal (and human) body weight. The time-weighted average body weight of the control mice (column 8 of Table 3) is taken as the body weight most representative of the overall body weight across doses. As discussed above, given the exponential nature of the body weight scaling formula and the relatively small differences in time-weighted average body weight across doses in the mice, the choice among the time-weighted dose-specific mouse body weights has a relatively small impact on the human equivalent dose and the corresponding soil remediation criterion. Column 9 of Table 4a-d gives the human equivalent dose (mg/kg/day) corresponding to a $1x10^{-6}$ lifetime cancer risk. Multiplying this dose by the assumed human body weight gives the corresponding mass of Cr^{+6} ingested daily (mg/day) which results in a 1×10^{-6} lifetime cancer risk.

The human lifetime cancer risk can be generalized by dividing the risk of $1x10^{-6}$ by the corresponding dose in column 9. The resulting value is the cancer potency slope $(mg/kg/day)^{-1}$), the risk for each mg/kg/day intake. This is given in column 10. For the slope derived from the male mouse data, the slope ranges from 0.3-0.5 $(mg/kg/day)^{-1}$, but all of the models except the probit model give a value of 0.5 $(mg/kg/day)^{-1}$. This range can be compared to the slopes of other well known chemicals that are carcinogenic by ingestion, such as benzo(a)pyrene, (7.3 $(mg/kg/day)^{-1}$), arsenic (1.5 $(mg/kg/day)^{-1}$), carbon tetrachloride (0.13 $(mg/kg/day)^{-1}$), dimethylnitrosamine (51 $(mg/kg/day)^{-1}$) (USEPA 2007a), with higher numbers corresponding to greater potency.

The NJDEP Soil Remediation Standards (final) (NJDEP, 2008) integrate the body weight from 1 year to 31 years of age in deriving soil cleanup standards for the ingestion/dermal pathway. This corresponds to a time-weighted average human body weight of 59 kg. The daily ingested intake of Cr^{+6} corresponding to a $1x10^{-6}$ lifetime human cancer risk (column 11 of Table 4a-d) is calculated by multiplying the human dose for this risk (column 9) by 59 kg.

Calculation of the Soil Concentration Corresponding to a $1x10^{-6}$ Lifetime Cancer Risk
The time-weighted averaging procedure for the amount of soil ingested daily that is specified in the NJDEP Soil Remediation Technical Regulations yields an integrated value of 114 mg/day for daily soil ingestion from 1 year to 31 years of age. Dividing the daily intake of Cr^{+6} corresponding to a $1x10^{-6}$ lifetime cancer risk (column 10) by the daily soil ingestion gives the concentration of Cr^{+6} in soil (mg Cr^{+6}/kg soil) corresponding to the $1x10^{-6}$ lifetime cancer risk (column **12** of Table 4a-d).

For a soil remediation criterion of 1 x 10^{-6} lifetime cancer risk, the dose-response models for the male mice all yield soil remediation criteria that converge very closely to a soil concentration of 1 mg Cr^{+6}/kg. It is noteworthy that the better fitting models in the combined data sets (Table 4c,d) also yielded the same soil remediation criterion values. Since most of the models (including the cancer multi-stage model preferred by USEPA) provide essentially equivalent fits to the data and yield the same soil remediation criterion value, it is not necessary to select a single model as the basis for the soil remediation criterion.

Weight of Evidence Considerations and Risk Characterization

Weight of evidence for characterization of carcinogenicity to humans by ingestion The results of the NTP study clearly show that ingestion of Cr^{+6} in drinking water resulted in tumors in both sexes of rats and mice. The database from the NTP study is judged to be of high quality. The study was well designed and well executed with no significant problems that raise questions about the validity of the results. Both the survival and the overall health of the animals were comparable to control animals at all doses with no clinical signs of toxicity. The decreased weight of the female mice at the highest dose (20% less than controls) may partly reflect systemic effects and, as such may indicate moderate exceedance of the maximum tolerated dose (MTD**)**.

The statistically significant increase in tumors in both rats and mice occurred in the alimentary system. In both the male and female mice, a clear dose-response was observable extending through the two highest doses. In the female mice, the response at the third highest dose was also increased consistent with the overall dose response. As discussed in Appendix A of this document, the evidence supports a hypothesis that the observed tumor incidence is relevant to human exposure at reasonably anticipated environmental levels, and did not occur due to exceedance of the gastrointestinal reduction capacity for Cr^{+6}.. Although the pH of the mouse stomach is higher than the pH of the human stomach, it appears that pH is not the predominant factor in the reduction of Cr^{+6} in the stomach, and that the mouse is a reasonable model for the carcinogenic potential of ingested Cr^{+6} in humans. Thus, the mode(s) of action of Cr^{+6} carcinogenicity responsible for the observed tumors in the mouse small intestine are likely to be relevant to the potential for carcinogenicity in the human gastrointestinal system. In addition, the observed carcinogenicity of Cr^{+6} by ingestion is consistent with the inhalation carcinogenicity of Cr^{+6} observed in studies of occupational exposure.

Under the current USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a), these observations are consistent with the characterization of oral exposure to Cr^{+6} as "likely to be carcinogenic to humans." More specifically, the data are consistent with the criterion for this characterization of "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans."

Weight of evidence for the carcinogenic mode of action (MOA) of Cr^{+6} -
There are considerable data indicating the ability of Cr^{+6} to react directly and indirectly with DNA including the production of mutations with *in vivo exposure* (Kirpnick-Sobol et al., 2006; Dana Devi et al., 2001; Cohen et al., 1993; Coogan et al., 1991; Knudsen et al., 1980; Itoh and Shimada, 1998). However, the data on the occurrence of diffuse hyperplasia in the mouse duodenum suggests that tissue damage and regeneration could have played a role in the formation of tumors in the mouse small intestine in the NTP study. The criteria for determination that a carcinogen operates through a mutagenic MOA with respect to the application of an of an age-dependent adjustment factor to the cancer potency (ADAF), as described in the USEPA's Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, are still under development. Thus, the age-dependent adjustment factor for carcinogens which act through a mutagenic MOA is not applied in this assessment.

Reliability of the quantitative procedure for calculating the cancer potency estimate and soil remediation criterion - Although the true shape of the dose-response data below the POD is not known, the cancer potency estimate (and associated soil remediation criterion) was derived from the NTP mouse data using a linear-from-POD approach. In the USEPA Guidelines for Carcinogen Risk Assessment (USEPA, 2005a), this is the default approach in the absence of sufficient evidence to support a non-linear mode of action. The fit of all of the mathematical models available in the BMDS package to the small intestine tumor data from the male mice was strong, and the difference between the BMD and BMDL doses was less then a factor of two for most of the models. Although the tumor data from the male mice were significantly better fit by the dose-response models than were data from the combined data sets, the best fitting models for the combined data sets resulted in the same potency estimates and soil remediation criterion values. Thus, the POD is statistically robust. The linear extrapolation procedure for the calculation of the cancer potency slope from the POD is entirely deterministic and requires no interpretation. Therefore, within the bounds of the default USEPA methodology, the quantitative cancer potency slope value derived here is judged to be robust and reliable.

The derivation of the ingestion-based remediation criterion value from the cancer potency estimate follows NJDEP-SRP procedures and exposure assumptions as specified in the Soil Remediation Standards (final) (NJDEP, 2008), and involves no additional data interpretation.

Characterization of uncertainty - While it seems clear that the oral cavity tumors in the rats and the small intestine tumors in the mice both resulted from ingestion of Cr^{+6}, it is unclear why there was a lack of concordance in the location of the tumors in these species. It should be noted, however, that the USEPA Guidelines for Carcinogen Risk Assessment (2005a) state that, "Target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans."

It is known that the gastrointestinal tract has a reserve capacity for the reduction of Cr^{+6} to Cr^{+3}. It has been argued that this capacity is sufficient to reduce the relatively large doses of Cr^{+6} that could reasonably be anticipated to be encountered under extreme conditions of environmental contamination (De Flora et al., 1987, 1997). It might, therefore, be hypothesized that the tumors observed in the NTP study reflect a threshold mechanism that functions only after this reduction capacity is exceeded, and that such a mechanism is not relevant to human environmental exposure. As discussed in detail in Appendix A of this document, analysis of the estimated maximum mouse intake rate of Cr^{+6} in comparison to the estimated reduction capacity of mouse gastric fluid suggests that even under the assumption that the mouse stomach is a closed system with respect to reduction of Cr^{+6}, the reduction capacity of the mouse gastrointestinal system would only potentially be exceeded at the highest dose in female mice. However, as explained further in Appendix A, however, the mouse stomach is not a closed system and the kinetics of gastric emptying, make it likely that even at very low doses, a significant fraction of ingested Cr^{+6} will reach the small intestine without being reduced. Even under the limiting assumption of a closed gastric reduction system, however, the possibility of exceedance of the reduction capacity at the highest dose in female mice cannot explain the overall dose-response pattern showing a significant increases in tumor incidence in both male and female mice at the two highest doses and a non-significant, but consistent increase in female mice at the third highest dose. A separate analysis of the rate of accumulation of Cr in various mouse tissues and biological media as a function of dose (see Appendix A) is, likewise, inconsistent with a hypothesis of a threshold for exceedance of the reduction capacity at the doses in the NTP study. In addition, the observation of diffuse hyperplasia in the duodenum of both sexes of mice at the lowest dose is also inconsistent with the exceedance of the reduction capacity at any dose in the NTP study. Overall, there is no evidence of a threshold for tumor production, including exceedance of the gastrointestinal reduction capacity of Cr^{+6} within the dose range of the NTP study. In addition, there are no data to support a hypothesis that assumes a threshold for exceedance of the Cr^{+6} reduction capacity at doses below those in the NTP study. Such a hypothesis is, furthermore, inconsistent with the evidence for a substantial reserve capacity of the gastrointestinal system for reduction of Cr^{+6} and also inconsistent with evidence of adverse systemic effects of ingested low dose Cr^{+6}.

NTP observed a decrease in hematocrit, particularly at the two highest doses in female mice (hematologic analysis was not carried out on male mice). Dehydration, would be expected to decrease blood fluid volume and, therefore, increase the hematocrit. NTP thus noted the decrease in hematocrit as evidence that dehydration did not occur in these groups despite significantly decreased body weight. The decrease in hematocrit was

accompanied by microcytosis. It, therefore, appears that this decrease in hematocrit resulted from a systemic effect of Cr^{+6}. It is theoretically possible that the systemic decrease in hematocrit could have masked a decrease in blood fluid volume from dehydration. However, the lack of physical signs of dehydration argues against this possibility.

Based on kinetic, chemical, and toxicological considerations, it appears that the doses of Cr^{+6} in the NTP study did not exceed the reduction capacity of the gastrointestinal system. It also appears likely that even at lower doses, a significant fraction of ingested Cr^{+6} will escape reduction in the gastrointestinal tract. This raises the possibility that ingested Cr^{+6} could cause tumors at sites distant from the gastrointestinal tract. There was no evidence of such tumors, however, in the NTP study. Furthermore, although the Davidson et al. (2004) study suggested that ingested Cr^{+6} could be a co-carcinogenic with UV light in the production of skin tumors, there is currently no evidence in the literature of non-gastrointestinal cancer resulting solely from Cr^{+6} ingestion. Blood is known to have a significant reduction capacity for Cr^{+6} as do other organs (De Flora et al., 1997). Nonetheless, the potential for ingested Cr^{+6} to cause tumors at other locations remains an uncertainty.

The USEPA default procedure for calculation of cancer potency that was employed herein linearly extrapolates across 5 orders of magnitude of cancer incidence from the data-based benchmark incidence rate (BMR) of 0.1 to estimate the dose at 1×10^{-6} (one-in-a million) cancer incidence. The shape of the dose-response function is not known below the range of the observed data, and the linear extrapolation across so large a range carries significant uncertainty. Although at the present time, there is no way to further reduce this uncertainty, this derivation of cancer potency for Cr^{+6} is entirely consistent with the approaches used for other cancer potency estimates calculated according to USEPA methodology.

Comparison of the suggested and current soil remediation criteria- The bases for the suggested soil remediaton criterion for Cr^{+6} ingestion derived from the NTP data (1 ppm) and the soil remediation criterion for Cr^{+6} inhalation cancer risk (20 ppm) are essentially unrelated. They reflect different conditions and routes of exposure and are supported by different cancer endpoints in different organs that may result from different toxicological modes of action.

Other ingestion cancer risk assessments based on the NTP data - The USEPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed a cancer potency based on the NTP chronic bioassay. This is discussed in Appendix B.

References

Beaumont JJ, Sedman RM, Reynolds SD, Sherman CD, Li LH, Howd RA, Sandy MS, Zeise L, Alexeeff GV (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. Epidemiology. 19:12-23.

Borneff J, Engelhardt K, Griem W, Kunte H, Reichert J (1968). Carcinogenic substances in water and soil. Archiv Hygiene Bakteriol. 152:45-53 (German).

Cohen MD, Kargacin B, Klein CB, Costa M (1993). Mechanisms of chromium carcinogenicity and toxicity. Crit Rev Toxicol. 23:255-81.

Coogan TP, Motz J, Snyder CA, Squibb KS, Costa M (1991). Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rats to potassium chromate. Toxicol Appl Pharmacol. 109:60-72.

Dana Devi K, Rozati R, Saleha Banu B, Jamil K, Grover P (2001). In vivo genotoxic effect of potassium dichromate in mice leukocytes using comet assay. Food Chem Toxicol. 39:859-65.

Davidson T, Kluz T, Burns F, Rossman T, Zhang Q, Uddin A, Nadas A, Costa M (2004). Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. Toxicol Appl Pharmacol. 196:431-7.

De Flora S, Badolati GS, Serra D, Picciotto A, Magnolia MR, Savarino V (1987). Circadian reduction of chromium in the gastric environment. Mutat Res. 1923:169-74.

De Flora S, Serra D, Camoirano A, Zanacchi P (1989). Metabolic reduction of chromium, as related to its carcinogenic properties. Biol Trace Elem Res. 21:179-87.

De Flora S, Camoirano A, Bagnasco M, Bennicelli C, Corbett GE, Kerger BD (1997). Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity. Carcinogenesis. 18:531-7.

IARC (2007). Overall Evaluations of Carcinogenicity to Humans - List of all agents evaluated to date
Accessed at: http://monographs.iarc.fr/ENG/Classification/crthalllist.php, 7/24/07.

Itoh S, Shimada H (1998). Bone marrow and liver mutagenesis in lacZ transgenic mice treated with hexavalent chromium. Mutat Res. 412:63-7.

Kerger BD, Richter RO, Chute SM, Dodge DG, Overman SK, Liang J, Finley BL, Paustenbach DJ (1996a). Refined exposure assessment for ingestion of tapwater contaminated with hexavalent chromium: consideration of exogenous and endogenous reducing agents. J Expo Anal Environ Epidemiol.6:163-79.

Kirpnick-Sobol Z, Reliene R, Schiestl RH (2006). Carcinogenic Cr(VI) and the nutritional supplement Cr(III) induce DNA deletions in yeast and mice. Cancer Res. 66:3480-4.

Knudsen I. (1980). The mammalian spot test and its use for the testing of potential carcinogenicity of welding fume particles and hexavalent chromium. Acta Pharmacol Toxicol (Copenh) 47:66-70.

NJDEP (2006). NJDEP Chromium Workgroup, Report for Public Comment and Response to Peer Review Comments. Accessed at: http://www.state.nj.us/dep/dsr/chromium/report.htm, 7/24/07.

NJDEP (2008). Ingestion-Dermal Exposure Pathway. Soil Remediation Standards **Basis And Background. June, 2008.** **http://www.nj.gov/dep/srp/regs/srs/bb_ingest_dermal.pdf.**

NTP (National Toxicology Program) (2008a). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate In F344/N Rats and B6C3F1 Mice. July 2008, National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: http://ntp.niehs.nih.gov/files/546_web_FINAL.pdf 8/6/08.

Petrilli FL, de Flora S (1988). Metabolic reduction of chromium as a threshold mechanism limiting its in vivo activity. Sci Total Environ. 71:357-64.

Rodricks JV, Gaylor DW, Turnbull D (2001). Quantitative Extrapolations in Toxicology, in *Principles and Methods in Toxicology*, 4 ed. Hayes WA, editor. CRC Press, Boca Raton, FL.

Uddin AN, Burns FJ, Rossman TG, Chen H, Kluz T, Costa M (2007). Dietary chromium and nickel enhance UV-carcinogenesis in skin of hairless mice. Toxicol Appl Pharmacol. 221:329-38.

USEPA (2003). Toxicological Review of dichloroacetic acid, EPA 635/R-03/007.

USEPA (2004). Toxicological Review of 1,2-dibromomethane**,** EPA 635/R-04/067.

USEPA (2005a). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F.

USEPA (2005b). Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. EPA/630/R-03/003F.

USEPA (2007a). Integrated Risk Information System (IRIS), accessed at: http://www.epa.gov/iriswebp/iris/subst/0144.htm, 7/24/07.

USEPA (2007b). Benchmark Dose Software, accessed at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=164443, 6/15/07.

USEPA (2007c). Glossary of IRIS terms, accessed at: http://www.epa.gov/iriswebp/iris/gloss8.htm#b, 6/15/07.

Appendix A

Issues Relating to the Capacity of the Gastrointestinal Tract to Reduce Cr^{+6}

It has been known for some time, based on *in vitro* studies with human gastric fluid, that reduction of at least some portion of ingested Cr^{+6} to Cr^{+3} occurs in the human stomach (De Flora et al., 1987, 1997). However, no data on the capacity of the stomach of rodents to reduce chromium is available. De Flora et al. (1997) estimated that the total Cr^{+6} reduction capacity of human gastric fluid is 10 mg between meals and up to 35 mg in the 2-4 hours following meals. Based on this, they have argued that the reduction capacity of the human stomach is sufficiently large to maintain an excess reduction capacity even with high doses of Cr^{+6}. Given this assertion, it is appropriate to ask whether the observed increase in intestinal tumors at the doses employed in the NTP study results from an exceedance of the reduction capacity of the mouse gastrointestinal system. If the tumors occur only because the reductive capacity of the mouse stomach was exceeded, they may be less relevant to human risk at the lower doses that are more likely to be encountered under environmental conditions.

The relative gastrointestinal absorption of Cr^{+6} and Cr^{+3}

Based on their relative levels in urine or organs following oral or gastric administration, it is known that Cr^{+6} is absorbed more readily from the gastrointestinal tract of rodents than Cr^{+3} by a factor of about 1.8-60 in different studies (Donaldson and Barreras, 1966; Maruyama, 1982; MacKenzie et al., 1959). This mirrors the difference in absorption between Cr^{+3} and Cr^{+6} in humans, based on area-under-the-curve for urinary Cr, by a factor of 53 (Kerger et al.,1996b). NTP conducted a 2-year bioassay with the Cr^{+3} dietary supplement, chromium picolinate, formulated to maximize the generally low bioavailability of Cr^{+3} (NTP, 2008b). This bioassay was conducted in parallel with NTP's study of sodium dichromate and employed the same strains of rats and mice.

As part of that study, the concentration of total Cr that was retained in plasma, erythrocytes, liver, kidney, glandular stomach and forestomach of male rats and female mice was measured at 25 weeks. This concentration was compared for similar doses of Cr^{+3} and Cr^{+6} (15.18 and 8.95 mg/kg/day as Cr, respectively in male rats; and 36.73 and 13.2 mg/kg/day as Cr respectively in female mice). These data are presented, in part, in Fig. 7 of the NTP final report for sodium dichromate dihydrate (NTP 2008a) and were supplemented by personal communication from NTP.[2] Despite the fact that the Cr^{+3} dose was 1.8 and 2.8 times larger than the Cr^{+6} dose in rats and mice respectively, the concentration of total Cr in these tissues was 1.4-16.7 times larger for the rats ingesting Cr^{+6}, and 2.1-38.6 times larger for the mice ingesting Cr^{+6}. The lower end of these ranges was found in the plasma, which was inconsistent with the Cr concentrations found in the other tissues. The reason for this difference between plasma and the other tissues is that there was a 48 hr "washout period" between the end of dosing and the collection of the tissue samples. Cr was largely cleared from the plasma during this period and the remaining Cr in the plasma mainly reflects redistribution from other tissues. For the other tissues, the Cr concentration was 5.3-16.7 and 6.6-38.6 times larger for the rats and

[2] Dr. Mathew Stout, NTP - personal communication 3/23/09.

mice (respectively) ingesting Cr^{+6} compared to animals ingesting Cr^{+3}. It seems clear that despite the assumed capacity of the gastrointestinal tract to reduce Cr^{+6}, at least at this dose, ingested Cr^{+6} was absorbed as Cr^{+6} rather than Cr^{+3}.

Do the NTP pharmacokinetic data provide evidence that the Cr^{+6} reduction capacity of the mouse gastrointestinal tract was exceeded?

If the reduction capacity of the mice was exceeded at the higher Cr^{+6} water concentrations of sodium dichromate that were also associated with increased intestinal tumors, there would be a threshold concentration at which unreduced Cr^{+6} would become available for absorption. Given the significantly greater rate of Cr^{+6} absorption, such a threshold would be evidenced by an increased rate of accumulation of total Cr in the blood and organs. An increased rate of absorption in conjunction with a threshold concentration would appear as a positive change in the slope of tissue Cr concentration versus drinking water concentration. This hypothesis can be investigated using the detailed animal-specific data, summarized in Appendix J, "Chromium Tissue Distribution Study" of the NTP 2-year bioassay (animal-specific data provided as a personal communication[1]), as well as data in the NTP short-term toxicokinetic study (NTP, 2007) conducted in conjunction with its 2-year bioassay. In the first study, female mice from among the exposure groups in the overall chronic bioassay were sacrificed at different time points and the total Cr concentration in various tissues and biological media was measured. In the second study, 6-7 week old male mice (the same strain used in the 2-year study) were provided with drinking water *ad libitum* containing 1, 3, 10, 30, 100 or 300 mg/L Cr^{+6} as sodium dichromate dihydrate for 21 days. Animals were sacrificed and total Cr concentration was measured in blood and kidney. Figure A-1a-g presents these data for female mice for all drinking water concentrations. Figure A-2 presents the data from the 21-day study in male mice.

We investigated this hypothesis through statistical analysis of the NTP pharmacokinetic data. For the female mouse data, this hypothesis was tested for each of the tissues and biological media by determining whether the slope of a linear function of tissue Cr concentration versus water concentration of Cr^{+6} fit to a portion of the data was significantly different from the linear slope fit to the entire data set. The portions of the data set from 0 mg/L sodium dichromate water concentration to the first concentration (14.3 mg/L) and from 0 mg/L to the second concentration (57.3 mg/L) were selected for this test based on examination of the full data set. The hypothesis was tested at each of the four time points at which tissue Cr concentrations were determined. For all tissues and biological media, there was no significant difference between the slope for the partial data set and the slope of the full data set at any of the time points. For the male mouse data, it is clear from visual examination of Fig. A-2 that the trend of Cr accumulation with increasing dose is supralinear (i.e. convex) across all doses. That is, changes in the slope reflect a decrease in the rate of Cr accumulation with increasing dose rather than the increase that would be expected if there were an exceedance of the reductive capacity of the gastrointestinal tract. These findings do not support the hypothesis that the reduction capacity of the mouse gastrointestinal tract was exceeded at some dose in the NTP study. In this respect, it is interesting to note that diffuse hyperplasia was seen in

[1] Dr. Bradley Collins, personal communication, 6/18/08

the duodenum at all doses in both sexes of mice. NTP attributed these lesions to regenerative cell growth secondary to tissue injury. It is not clear whether this response is causally related to the development of the observed neoplasms. This response is, nonetheless, associated with Cr^{+6} exposure. The lack of an observed threshold for this response, including at the lowest doses where the reductive threshold is not expected to be exceeded, likewise does not support the hypothesis that the mice exceeded a threshold for reduction of Cr^{+6} in this study.

Comparison of Cr^{+6} intake and the reduction capacity of mouse gastric fluid

Another approach to evaluating whether the Cr^{+6} exposures in the NTP study overwhelmed the reductive capacity of the mice is to assess their estimated reduction capacity compared to their rate of Cr^{+6} intake. There are no data on the reduction capacity of mouse gastric fluid. However, the reduction capacity of mouse gastric fluid can be estimated from data on the reduction capacity of human gastric fluid. Based on experiments in which aspirated human gastric fluid was reacted *ex-vivo* with Cr^{+6}, and data on the total daily volume of human gastric fluid, De Flora et al. (1987, 1997) estimated the Cr^{+6} reduction capacity of human gastric fluid at >84-88 mg Cr^{+6}/day, although they also indicate that the procedures used in preparation of the gastric fluid likely resulted in a underestimation of its reductive capacity (De Flora et al., 1997). They also estimated an additional 11-24 mg/day reduction capacity from intestinal bacteria, but it is unclear how much of this capacity resides in the small intestine. Ingested Cr^{+6} is not resident in the stomach for an entire day, but is likely to be either rapidly absorbed (see below) or passed on to the small intestine as a function of gastric emptying time ($T_{1/2}$ for gastric emptying in humans is reported as 127 min by Hellmig et al.,2006). De Flora et al. (1997) reported that the reduction capacity of gastric fluid reached a maximum in conjunction with meals and was sustained for 2-3 hr following the meal. This is consistent with the above estimate for $T_{1/2}$ for gastric emptying. The meal-associated reduction was 25.1 mg Cr^{+6}/meal. This gives a meal-associated reduction rate of 25.1 mg Cr^{+6}/2.5 hr = 10 mg Cr^{+6}/hr. This value can be scaled to the mouse gastric fluid on the basis of $(\text{body-weight})^{3/4}$. This method of interspecies scaling takes metabolic and physiological factors (e.g., food consumption rate, gastric fluid secretion rate) into account. While the actual chemical process governing the reduction of Cr^{+6} is probably a physico-chemical interaction and thus not a function of body weight, the circumstances governing the conditions under which this chemical interaction occurs are likely to be under metabolic and physiological control. These include the production and secretion of the chemicals involved in the reduction reaction, the gastric mixing, and the gastric and small intestine emptying time. Therefore, $(\text{body-weight})^{3/4}$ scaling was applied in the calculation of the human cancer potency slope in Table 3 of the main document. Using the human reduction rate of 10 mg Cr^{+6}/hr and assuming an adult human body-weight of 70 kg, adjustment on the basis of $(\text{body-weight})^{3/4}$ gives a generalized $(\text{body-weight})^{3/4}$ gastric fluid reduction rate which should be applicable to any mammalian species of 0.4132 mg/hr/$kg^{3/4}$. This rate can be applied to the mice in the NTP study by multiplying by the control mouse $(\text{body weight})^{3/4}$ (i.e., $(0.050)^{3/4}$ and $(0.053)^{3/4}$ for males and females respectively). This gives values of 0.044 mg/hr and 0.046 mg/hr for males and females.

The Cr^{+6} intake rate of the mice can be estimated from the rate of water consumption in mice. Ho and Chin (1988) reported that daily water consumption in mice was 4.4 +/- 0.3 ml. Water consumption was closely linked temporally to eating, and 86% of water consumption occurred during the 12 hr dark period. Thus, the hourly dark period water consumption rate can be estimated as (4.4 ml x 0.86)/12 hr or 0.32 ml/hr. Toya and Clapp (1972) measured 5.7 ml of water consumption by mice during a 17 hr overnight period giving a nighttime consumption rate of 0.34 ml/hr. Given this close agreement, the average rate of 0.33 ml/hr is assumed. This rate of water consumption for the maximum period of water intake (i.e., night), can be multiplied by the Cr^{+6} drinking water concentrations in the NTP study to give an estimate of the maximum rate of Cr^{+6} intake for the mice (mg/hr). The estimated maximum Cr^{+6} intake rate for the mice at each dose is presented in Table A-1.

Comparing the estimated capacity of gastric reduction of Cr^{+6} of 0.044 and 0.046 mg/hr for male and female mice respectively, to the estimated maximum Cr^{+6} intake rates in Table A-1, shows that only the intake rate for female mice at the highest concentration of Cr^{+6} in drinking water (0.059 mg/hr) exceeds the estimated reduction capacity. The next highest intake rate in female mice is 43% of the estimated reduction capacity. The highest intake and second highest rate in male mice were 68% and 23% of the estimated reduction capacity. While only the highest dose in females exceeded the estimated reduction capacity in this analysis, the observed significant increase in tumor rates occurred at the two highest doses in males and the two highest doses in female mice (along with a non-significant, but consistent increase at the third highest dose). Therefore, the observed increase in tumor rates is not consistent with the hypothesis that the mouse intestinal tumors resulted from the intake rate of Cr^{+6} exceeding the reduction capacity.

Table A-1

Estimated Cr^{+6} intake rates for male and female mice as a function of Cr^{+6} drinking water concentration

Cr^{+6} water conc. (mg/L)	**Mean peak period Cr^{+6} intake rate (Assuming water consumption at 0.33 ml/hr)** (mg/hr)
Male mice	
0	0
5	0.0017
10	0.0033
30	0.0099
90	0.030
Female mice	
0	0
5	0.0017
20	0.0066
60	0.020
180	0.059

This comparison assumes that with respect to the reduction of Cr^{+6}, the mouse stomach can be viewed as a closed system. That is, that the mass of ingested Cr^{+6} is all present in the stomach during the entire period under consideration. In fact, even if the reduction capacity of the mouse stomach is not exceeded, the extent of reduction is limited by the kinetics of gastric emptying. The half-time for gastric emptying of liquids in the mouse has been reported as <5-9 min (Moretó et al., 1982; Symonds et al., 2007). This means that even when the hourly rate of Cr^{+6} reduction greatly exceeds the hourly rate of Cr^{+6} intake, a substantial fraction of the ingested Cr^{+6} can be expected to escape reduction by being transported from the stomach to the small intestine.

Effect of pH on reduction capacity for Cr^{+6}

There is a difference in the pH of human and mouse gastric fluids. In the normal human gastric fluid evaluated by De Flora et al. (1987, 1997), the pH varied from approximately 1.0-3.5. In the mouse, the pH of the stomach varies from 3.1-4.5 (Kararli, 1995; McConnell et al., 2008). While there is some relationship between pH and Cr^{+6} reductive capacity, the correlation is only moderate. This can be seen in the study of De Flora et al. (1987) in which aspirated gastric fluid from individuals collected hourly for 24 hours was reacted with Cr^{+6}. These individuals included those with normal gastric fluid as well as those with gastric fluid with higher than normal pH resulting from anti-secretory

medication or duodenal reflux. In normal subjects, the pH of the gastric fluid increased at the start of a meal from the fasting pH of approximately 1.0 to 3-3.5, probably due to the buffering capacity of the food. While the pH at the time of maximum reduction was approximately 1.5-2.0, the increased pH associated with the meal resulted in only about a 50% decrease in the reduction capacity (about 6 times the fasting capacity). Increasing the pH to 7.0 from an initial value of 0.8-1.5 resulted in a decrease in reduction by a factor of approximately 5. However, decreasing the pH to 1.0 from subjects with an initial pH of 5.1-7.2 resulted in only a slight and non-statistically significant increase in reduction. Thus, it appears that reduction capacity is affected to some extent by pH, but is largely under control of other gastric factors associated with eating, possibly including gastric secretions and food itself. Reduction appears to be accomplished by small molecules such as ascorbate rather than by thermo-labile substances such as enzymes (De Flora et al., 1987). Therefore, it seems unlikely that Cr^{+6} reduction capacity is significantly affected by potential inter-species differences in enzyme type or function or pH. Rather, it seems likely that interspecies differences in Cr^{+6} reduction capacity are a function of their underlying metabolic rate.

Additional evidence of the role of pH on the reduction capacity of gastric juice is provided by Kerger et al. (1996a). Cr^{+6} (5 mg) added to 10 ml of orange juice with a pH of 3.74 was 100% reduced within 15 minutes. In 10 ml of lemonade, with a pH of 3.01, 40% of 5 mg of Cr^{+6} was reduced in approximately 170 minutes. The difference in reduction in these two solutions, with the lower pH resulting in significantly less reduction, illustrates that the chemical nature of the gastric fluids rather than their pH is the critical factor in determining the extent of Cr^{+6} reduction. In addition, it should be noted that the orange juice, whose pH is within the range of the mouse stomach (3.1-4.5), rapidly and completely reduced a relatively large mass of Cr^{+6}. These lines of reasoning suggest that the validity of the above comparison of the Cr^{+6} reduction rate of human gastric fluid and the intake rate of Cr^{+6} in the NTP mice is not dependent on the pH difference between the human and mouse stomachs. Therefore, it is reasonable to assume that differences between humans and mice in the reduction capacity of their gastrointestinal tracts stem largely from differences in their metabolic rates rather than from differences in pH or biochemistry. Thus, inter-species differences in this function are appropriately adjusted on the basis of a $(\text{body-weight})^{3/4}$ adjustment.
When the metabolic rate is adjusted on this basis, the mouse appears to be a reasonable model for the human gastrointestinal carcinogenicity of Cr^{+6}.

Comparison of the reduction capacity of the mouse and rat

The rats exposed in the NTP study had an elevated incidence of tumors in the oral cavity but not in the small intestine or elsewhere in the gastrointestinal tract. This raises the possibility that the absence of an elevated incidence of gastrointestinal tumors in the rat results from a more efficient capacity for reduction of Cr^{+6} in the rat gastrointestinal tract than in the mouse. This can be investigated by comparing the rate of increase of Cr in urine as a function of Cr^{+6} drinking water concentrations in male rats and female mice at day 371. Urine is an appropriate medium for this comparison since urine integrates the total body absorption of Cr. Day 371 reflects the maximum accumulation for both species. The NTP data on accumulation of Cr do not permit the comparison of these data

for the same sex in rats and mice. For rats and mice, the linear slope of the relationship between mean urine Cr concentration and drinking water concentration is 3.00 and 0.94 μg Cr/g urine per mg Cr/L drinking water respectively. In other words, the rate of uptake of Cr from the rat gastrointestinal tract as a function of concentration of Cr^{+6} in drinking water is more than 3 times that of the mouse. Since the tumors in the mouse small intestine must have resulted from the absorption of Cr^{+6} into the intestinal tissue, the even greater rate of absorption of Cr by the rat must, likewise, reflect an even greater exposure of the intestinal tissues to Cr^{+6}. Thus, while it is not clear why the mice, but not the rats developed gastrointestinal tract tumors, the evidence does not support the hypothesis that the lack of gastrointestinal tumors in the rats reflects a more efficient reduction capacity for Cr^{+6}.

Human gastric reduction capacity and exposure to Cr^{+6}

The lack of evidence to support the hypothesis that the observed tumor incidence results from exceedance of the reduction capacity of the mouse gastrointestinal tract raises the question of whether similar considerations would also apply to human environmental exposures.. O'Flaherty et al. (2001) analyzed the data from a series of related studies of intentional human exposure to Cr^{+6} in drinking water (Paustenbach et al., 1996; Kerger et al., 1996a, 1996b; Finley et al., 1997) in which the daily Cr^{+6} dose ranged from 0.001 mg/kg to approximately 0.2 mg/kg. In one of these studies (Kerger, 1996b), both Cr^{+3} and Cr^{+6} (5 mg each) were ingested each by four subjects (with one subject separately ingesting both) and the rate of appearance of each in the urine (as total Cr) was compared. Consistent with the studies discussed above, both the rate of appearance and the overall area-under-the-curve of total Cr in urine were much larger for the ingestion of Cr^{+6} than for ingestion of Cr^{+3}. For Cr^{+3}, the mean peak urinary concentration was 8.9 μg/g creatinine and a total of 0.13% of the dose was recovered in the urine. For Cr^{+6}, in contrast, the peak concentration was 209 μg/g creatinine and 6.9% of the dose was recovered in the urine. With reference to this series of human dosing studies, O'Flaherty et al. (2001) concluded that based on the reduction capacity estimated by De Flora et al. (1997), "Even if all of the maximum single or multiple 5-mg doses had been ingested instantaneously, the total reducing capacity of gastric juice should not have been exceeded. Nonetheless, it is clear, based on total urinary chromium excretion, that a consistently greater percentage of the Cr^{+6} than of the Cr^{+3} was absorbed. This observation, consonant with other observations in humans (Donaldson and Barreras, 1966), implies that some Cr^{+6} escaped reduction in the stomach and entered portal venous blood. The greater absorption of Cr^{+6} than Cr^{+3} does not imply that the reduction capacity of gastric juice was exceeded, but rather that absorption from the gastrointestinal tract is so rapid that it is able to compete effectively with reduction in the stomach." This implies that, regardless of the reductive capacity of human gastric fluid, the kinetics of Cr^{+6} uptake from the gastrointestinal tract favor absorption of at least a portion of an ingested dose. The rapid uptake of Cr^{+6} compared with Cr^{+3} appears to result from the transport of anionic, Cr^{+6}-containing, chromate or dichromate complexes across cell membranes by the general anion transport system that is also responsible for transport of SO_4^{-2} and PO_4^{-3} (Cohen et al., 1993). Cr^{+3}, on the other hand, crosses cell membranes only by passive diffusion. Thus, whether Cr^{+6} is absorbed directly from the stomach as suggested by the epidemiologic data of Beaumont et al. (2008), or from the small

intestine as observed in the mice in the NTP study, the evidence strongly suggests that, as in the mouse, in exposed humans there can be significant exposure to unreduced Cr^{+6} even at low doses. This is consistent with the previously cited data on the kinetics of gastric emptying in mice.

Kerger et al. (1996a) proposed an alternative explanation for the more rapid and complete appearance of Cr in blood following Cr^{+6} ingestion compared to Cr^{+3} ingestion. They proposed that Cr^{+6} is reduced in the stomach to an organic complex that is a particularly absorbable form of Cr^{+3} . However, the literature cited in support of the existence of such complexes (Ronai, 1969; Edel and Sabbioni, 1985; Gargas et al., 1994; Kortenkamp and Beyersmann, 1987) does not address the formation of Cr^{+3} complexes in the gastrointestinal tract and/or does not establish the existence of an organic Cr^{+3} complex with absorption characteristics similar to Cr^{+6}. One of the studies cited (Gargas et al., 1994), examined the appearance of Cr^{+3} ingested as chromium picolinate, a synthetic organic nutritional supplement designed to maximize the otherwise low bioavailability of Cr^{+3}. Gargas et al. (1994) report the bioavailability of Cr^{+3} from chromium picolinate as 2.8%. Even given the attempt to maximize Cr^{+3} gastrointestinal uptake through the use of this organic complex, the bioavailability of Cr^{+3} is still considerably smaller than the value of 6.9% reported by Kerger et al. (1996b) for ingested Cr^{+6}. Another study (Gonzalez-Vergara et al., 1981) also employed novel synthetic (pyridoxilidene and nicotinic acid) complexes with Cr^{+3} with no indication that such complexes are produced in the gastrointestinal tract. Levis et al. (1978), who are also cited in support of this explanation, hypothesize absorption of Cr^{+3} due to stable "chelates and coordination complexes" formed in cell culture environments. However, they also note that internal cell concentrations of Cr resulting from incubation with soluble Cr^{+3} are 20 times lower than those resulting from the same concentration of Cr^{+6}. Mertz (1969, 1971) notes that the formation of coordination complexes with small molecules in the intestine and intake of Cr already bound to glucose tolerance factor make Cr available for absorption from the intestines. However, these observations do not distinguish between Cr^{+3} and Cr^{+6} in this regard, nor do they suggest that their absorption is comparable. Mertz (1969) also notes that Cr binds selectively to siderophilin, which facilitates its transport to tissues. Here, again, the valence of Cr is not specified and this binding is identified as a phenomenon in serum rather than in the intestines. O'Flaherty et al. (2001) conclude that the explanation of the formation of such Cr^{+3} complexes " … is considered implausible, because no known complexes of CrIII are absorbed to the extent that CrVI is."

Specific evidence against the hypothesis of intestinal uptake of Cr^{+3} as a readily absorbed organic complex is provided by Donaldson and Barreras (1966) who incubated Cr^{+6} in human gastric fluid (pH 1.4) for 30 minutes and then perfused the material into the jejunum of human subjects. Whereas approximately 25% of Cr^{+6} perfused into the jejunum without pre-incubation in gastric fluid was absorbed, the gastric fluid incubation resulted in nearly complete inhibition of absorption from the jejunum. This provides direct evidence that reduction of Cr^{+6} in the stomach does not result in readily absorbable forms of Cr^{+3}.

Evidence for low dose gastrointestinal absorption of Cr^{+6} by mice

The assumption that, based on evidence from human studies, Cr^{+6} is absorbed from the gastrointestinal tract of mice more rapidly than it is reduced to Cr^{+3} is supported by reports of systemic effects of low oral doses of Cr^{+6}. Davidson et al. (2004) found that in hairless mice exposed to UV light, significantly more skin tumors were produced in the mice that also received 0.13 mg/L Cr^{+6} as potassium chromate in their drinking water. This concentration is only 3% of the lowest drinking water concentration in the NTP study. Murthy et al. (1996) found ultrastructural abnormalities in the ovaries of Swiss albino mice given 5 mg/L Cr^{+6} in their drinking water, the same concentration as the lowest concentration in the NTP study. Dana Devi et al. (2001) investigated the levels of single strand DNA breaks in leukocytes as reflected in the comet assay in mice administered a single oral dose of potassium dichromate. At the lowest dose, 0.59 mg/kg as potassium dichromate (0.21 mg/kg as Cr^{+6}), as well as at higher doses, there was a statistically significant increase in DNA breaks compared to controls as measured by the length of the comet tail. The lowest dose in that study (as Cr^{+6}) is about half the lowest daily dose received in the NTP study. These observations are consistent with the low dose uptake of Cr^{+6} from the gastrointestinal tract of mice, but they are not consistent with known systemic effects of Cr^{+3}.

Conclusions regarding reduction capacity

In summary, there does not appear to be any clear evidence to support a hypothesis that the tumors in the mouse small intestine resulted from the Cr^{+6} doses in the NTP chronic bioassay overwhelming the reduction capacity of the gastrointestinal tract. In contrast, both the pharmacokinetic data on Cr accumulation in the various organs and the comparison of the mouse Cr^{+6} intake rate to the Cr^{+6} reduction rate in human gastric fluid provide evidence that the observed tumor incidence in the mice cannot be explained by exceedance of the reduction capacity.

Appendix B

Cancer Potency Derivation Based on the NTP Sodium Dichromate Chronic Bioassay by USEPA Office of Prevention, Pesticides and Toxic Substances (OPPTS)

The USEPA OPPTS conducted a risk assessment and cancer potency derivation based on the NTP's sodium dichromate chronic bioassay in conjunction with its consideration of the pesticide reauthorization of copper-chromate-arsenic (CCA) treated wood (USEPA, 2008a, b). The narrative portion of the assessment concluded that, with respect to the 2005 USEPA Cancer Guidelines, Cr(VI) is "Likely to be Carcinogenic to Humans" based on the presence of oral mucosa and tongue tumors in male and female rats and tumors of the small intestine in male and female mice at doses that were adequate, but not excessive, to assess carcinogenicity. There is clear evidence that Cr(VI) is mutagenic and convincing evidence supporting a mutagenic mode of action." (USEPA, 2008a). The quantitative derivation of a cancer potency estimate was based on combined intestinal tumors (duodenum, jejunum, and ileum) in female mice using the linearized multistage model (Q_1*) and (body-weight)$^{3/4}$ scaling of doses. Based on this approach, they derived a human cancer potency estimate of 0.79 (mg/kg/day)$^{-1}$.

The approach followed by the USEPA OPPTS differs from the one followed in this document in several respects. The OPPTS chose a potency derived from female mice (as opposed to rats of either sex or to male mice) because that species and sex was the most sensitive. That is, it yielded the largest potency estimate. In contrast, the cancer potency estimate derived in this document is based on male mice. The choice of male mice for the assessment provided in this document was based on the observation that, although female mice yielded a slightly larger estimate of potency with some benchmark dose models, the overall fit of those models to the female mouse data were poor and would generally be considered unacceptable.

The OPPTS also chose to use the linearized multistage model to calculate the cancer potency slope directly from the fit of the data to that model. In contrast, this document used the approach recommended in the current USEPA Cancer Guidelines (USEPA, 2005a) that calls for the slope to be calculated from a straight line extending from the point-of-departure (POD) to the point corresponding to zero incremental dose-zero incremental response. The two approaches are not equivalent and it is unclear why OPPTS chose not to follow the current USEPA (2005) guidelines. Also, OPPTS assumed a weight of 30 g for the female mice for use in the allometric dose conversion from mouse to human. In this document, the body weight for males and females control mice (50 and 53 g, respectively) was used for the allometric dose conversion. The basis for the choice of 30 g by OPPTS is unclear given that the time-weighted average weights for these mice varied from 42-53 g depending on the dose. Finally, the OPPTS assessment identified a mutagenic mode of action for Cr^{+6} carcinogenicity by the oral route of exposure. At the present time, the criteria for this determination are not clear and the age dependent adjustment factor for mutagenic MOA was not used in the risk assessment presented in this document.

Despite these differences in approach and interpretation, it is interesting to note that the OPPTS cancer potency estimate based on female mice, 0.79 $(mg/kg/day)^{-1}$, and its potency estimate based on male mice, 0.65 $(mg/kg/day)^{-1}$ are close to the estimate of 0.50 $(mg/kg/day)^{-1}$ provided in this document. Both OPPTS and the risk assessment presented herein conclude that under the current USEPA Cancer Guidelines, Cr^{+6} is "likely to be carcinogenic to humans."

References – Appendices A and B

Beaumont JJ, Sedman RM, Reynolds SD, Sherman CD, Li LH, Howd RA, Sandy MS, Zeise L, Alexeeff GV (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. Epidemiology. 19:12-23.

Cohen MD, Kargacin B, Klein CB, Costa M (1993). Mechanisms of chromium carcinogenicity and toxicity. Crit Rev Toxicol. 23:255-81.

Dana Devi K, Rozati R, Saleha Banu B, Jamil K, Grover P (2001). In vivo genotoxic effect of potassium dichromate in mice leukocytes using comet assay. Food Chem Toxicol. 39:859-65.

Davidson T, Kluz T, Burns F, Rossman T, Zhang Q, Uddin A, Nadas A, Costa M (2004). Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. Toxicol Appl Pharmacol. 196:431-7.

De Flora S, Badolati GS, Serra D, Picciotto A, Magnolia MR, Savarino V (1987). Circadian reduction of chromium in the gastric environment. Mutat Res. 1923:169-74.

De Flora S, Camoirano A, Bagnasco M, Bennicelli C, Corbett GE, Kerger BD (1997). Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity. Carcinogenesis. 18:531-7.

Donaldson RM Jr, Barreras RF (1966). Intestinal absorption of trace quantities of chromium. J Lab Clin Med. 68:484-93.

Edel J, Sabbioni E (1985). Pathways of Cr (III) and Cr (VI) in the rat after intratracheal administration. Hum Toxicol. 4:409-16.

Finley BL, Kerger BD, Katona MW, Gargas ML, Corbett GC, Paustenbach DJ (1997). Human ingestion of chromium (VI) in drinking water: pharmacokinetics following repeated exposure. Toxicol Appl Pharmacol. 142:151-9.

Gargas ML, Norton RL, Paustenbach DJ, Finley BL (1994).
Urinary excretion of chromium by humans following ingestion of chromium picolinate. Implications for biomonitoring. Drug Metab Dispos. 22:522-9.

Gonzalez-Vergara E, Condia de Gonzalez B, Hegenauer J, Saltman P (1981). Chromium coordination compounds of pyridoxal and nicotinic acid: synthesis, absorption and metabolism. Israel J Chem. 21:18-22.

Hellmig S, Von Schöning F, Gadow C, Katsoulis S, Hedderich J, Fölsch UR, Stüber E (2006). Gastric emptying time of fluids and solids in healthy subjects determined by 13C breath tests: influence of age, sex and body mass index. J Gastroenterol Hepatol. 21:1832-8.

Ho A, Chin A (1988). Circadian feeding and drinking patterns of genetically obese mice fed solid chow diet. Physiol Behav. 43:651-6.

Kararli, TT (1995). Comparison of the GI anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. BioPharm & Drug Dispos. 16:351-380.

Kerger BD, Richter RO, Chute SM, Dodge DG, Overman SK, Liang J, Finley BL, Paustenbach DJ (1996a). Refined exposure assessment for ingestion of tapwater contaminated with hexavalent chromium: consideration of exogenous and endogenous reducing agents. J Expo Anal Environ Epidemiol. 6:163-79.

Kerger BD, Paustenbach DJ, Corbett GE, Finley BL (1996b). Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water. Toxicol Appl Pharmacol. 141:145-58.

Kortenkamp A, Beyersmann D, O'Brien P (1987). Uptake of chromium (III) complexes by erythrocytes. Toxicology and Environmental Chemistry. 14: 23–32.

Levis AG, Bianchi V, Tamino G, Pegoraro B (1978). Cytotoxic effects of hexavalent and trivalent chromium on mammalian cells in vitro. Br J Cancer. 37:386-96.

MacKenzie RD, Anwar RA, Byerrum RU, Hopper CA (1959). Absorption and distribution of Cr^{51} in the albino rat. Arch Biochem Biophysics. 79:200-215.

Maruyama, J (1982). The health effect of mice given oral administration of trivalent and hexavalent chromium over long-term. *Acta Scholae Medicinalis Universitatis in Gifu* 31, 25-46.

McConnell EL, Basit AW, Murdan S (2008). Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. J Pharm Pharmacol. 60:63-70.

Mertz W (1969). Chromium occurrence and function in biological systems. Physiol Rev. 1969 Apr;49(2):163-239.

Mertz W, Roginski EE (1971). "Chromium metabolism: the glucose tolerance factor." In: Mertz W, Cornatzer WE (eds) "Newer Trace Elements in Nutrition." New York: Marcel Dekker, pp 123–153.

Moretó M, Guzmán L, Díez A (1982). A pattern for gastric emptying in mice. Am J Physiol. 242:G333-6.

Murthy RC, Junaid M, Saxena DK (1996). Ovarian dysfunction in mice following chromium (VI) exposure. Toxicol Lett. 89:147-154.

NTP (National Toxicology Program) (2007). NTP Technical Report on the Toxicity Studies of Sodium Dichromate Dihydrate Administered in Drinking Water to Male and Female F344/N Rats and B6C3F1 Mice and Male BALB/c and *am3*-C57BL/6 Mice. January 2007 National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX72.pdf, 7/25/07.

NTP (National Toxicology Program) (2008a). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate In F344/N Rats and B6C3F1 Mice. July 2008, National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: http://ntp.niehs.nih.gov/files/546_web_FINAL.pdf 8/6/08.

NTP (2008b). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Chromium Picolinate Monohydrate in F344/N Rats and B6C3F1 Mice. Scheduled for Peer-Review February 27-28, 2008, National Institutes of Health Public Health Service U.S. Department of Health and Human Services. Accessed at: http://ntp.niehs.nih.gov/files/TR556board_webRev.pdf 3/16/09.

O'Flaherty EJ, Kerger BD, Hays SM, Paustenbach DJ (2001). A physiologically based model for the ingestion of chromium(III) and chromium(VI) by humans. Toxicol Sci. 60:196-213.

Paustenbach DJ, Hays SM, Brien BA, Dodge DG, Kerger BD (1996). Observation of steady state in blood and urine following human ingestion of hexavalent chromium in drinking water. J Toxicol Environ Health. 49:453-61.

Ronai PM (1969). The elution of 51Cr from labelled leukocytes--a new theory. Blood. 1969 Mar;33(3):408-13.

Symonds EL, Butler R, Omari TI (2007). A mouse model for assessing the impact of ingested nutrients on gastric emptying rate. Clin Exp Pharmacol Physiol34:132-3.

Toya RE, Clapp NK (1972). Estimation of water consumption by individual mice caged in groups. Lab Anim Sci. 22:709-11.

USEPA (2008a). Cancer Assessment Document – Evaluation of the carcinogenic potential of inorganic hexavalent chromium (Cr(VI)), 3/12/08, Cancer Assessment review committee Health Effects Divison Office of Pesticide Programs. Final.

USEPA (2008b). Cr(VI): Quantitative risk assessment (Q_1)* based on F344/N rat and B6CF1 mouse carcinogenicity dietary studies with ¾ interspecies scaling factor. Memo from Lori L. Bronsman, Statistician, Science Information Management Branch, Health Effects Division (7509P) to Jonathan Chen, Toxicologist, Risk Assessment and Science Support Branch, Antimicrobial Division (7510P), 2/5/2008. Office of Prevention, Pesticides and Toxic Substances.

Fig. 1.

Incidence of oral tumors in male rats (not adjusted for number of animals at-risk – see text)

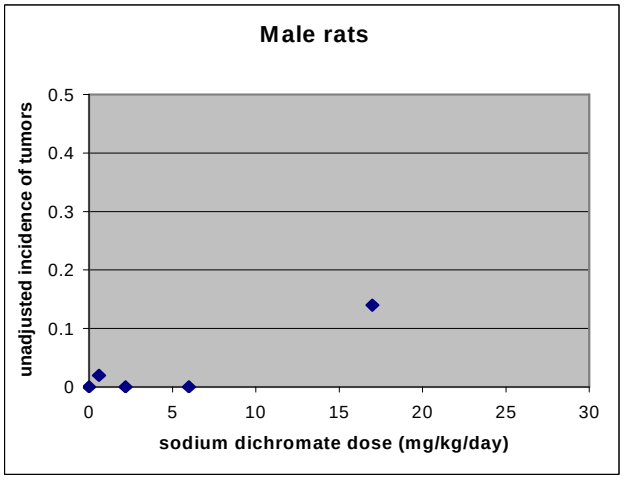


Fig. 2.

Incidence of oral tumors in female rats (not adjusted for number of animals at-risk – see text)

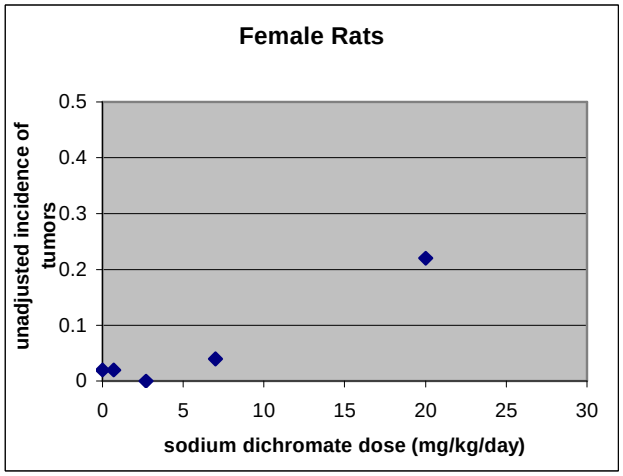


Fig. 3.

Incidence of intestinal tumors in male mice (not adjusted for number of animals at-risk – see text)

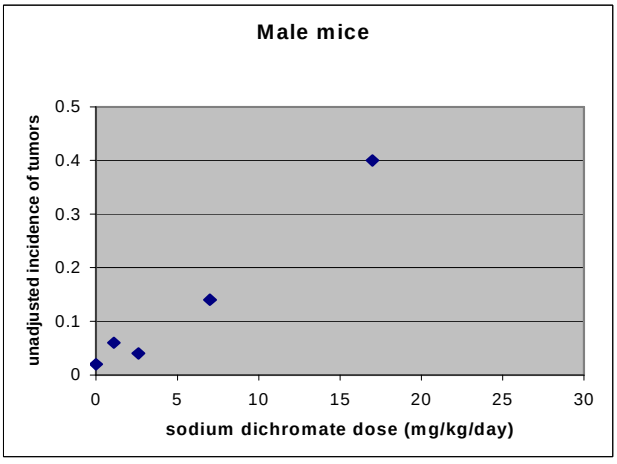


Fig. 4.

Incidence of intestinal tumors in female mice (not adjusted for of animals at-risk – see text)

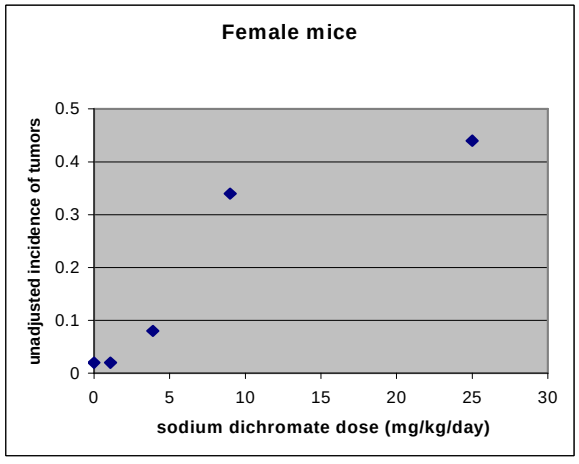
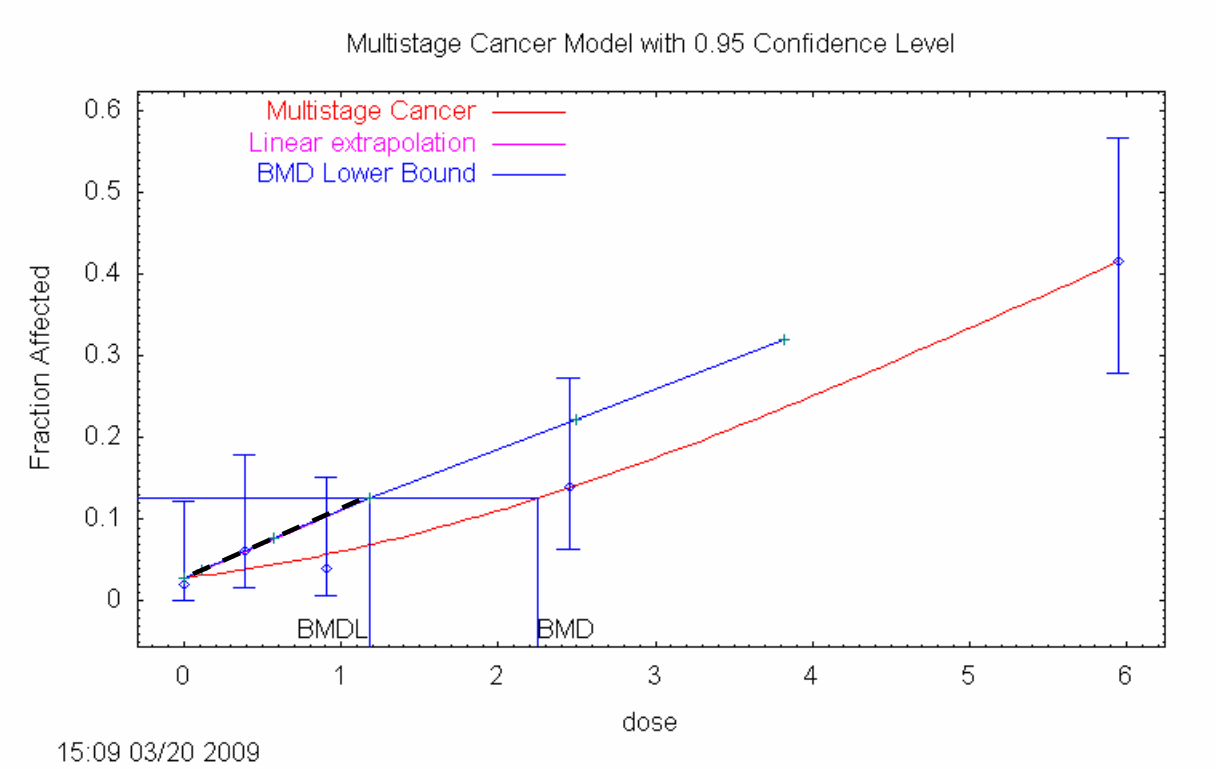


Fig. 5

Intestinal neoplasms in male mice benchmark dose modeling – Multistage cancer model

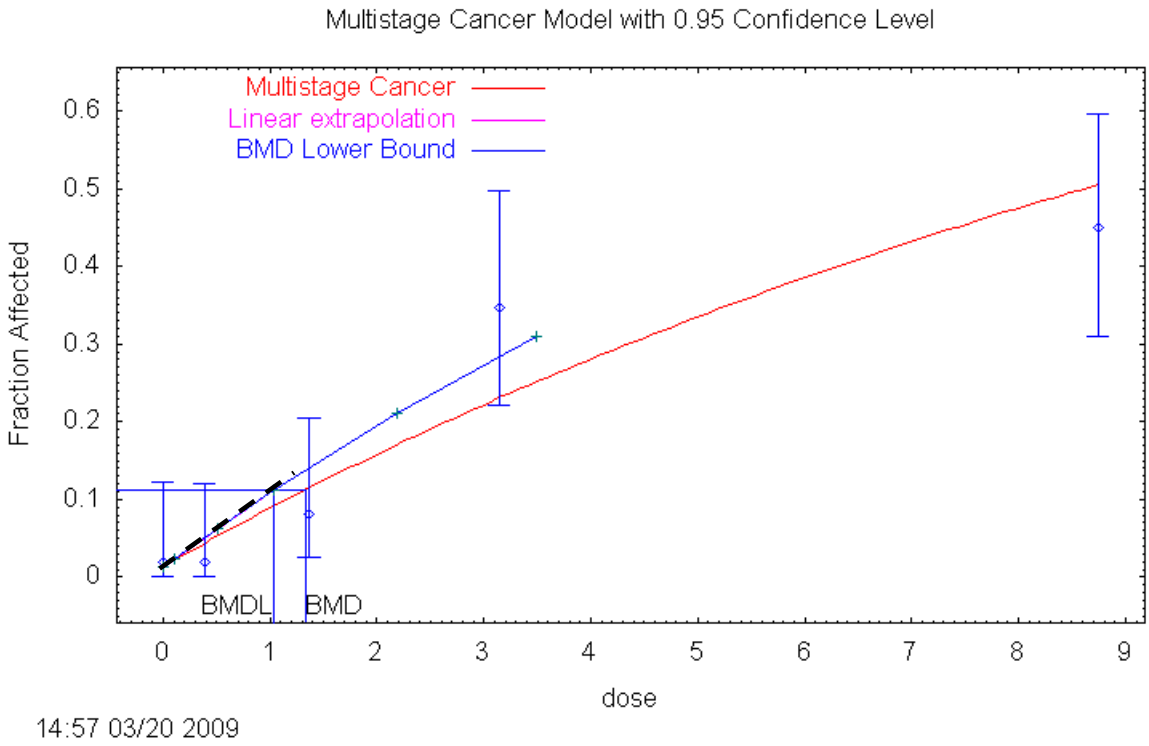


BMD (benchmark dose) – the dose corresponding to the 10% response rate after adjusting for response rate in controls

BMDL (benchmark dose-low) – the dose corresponding to the lower confidence bound on the 10% response rate after adjusting for response rate in controls

Fig. 6

Intestinal neoplasms in female mice benchmark dose modeling – Multistage cancer model

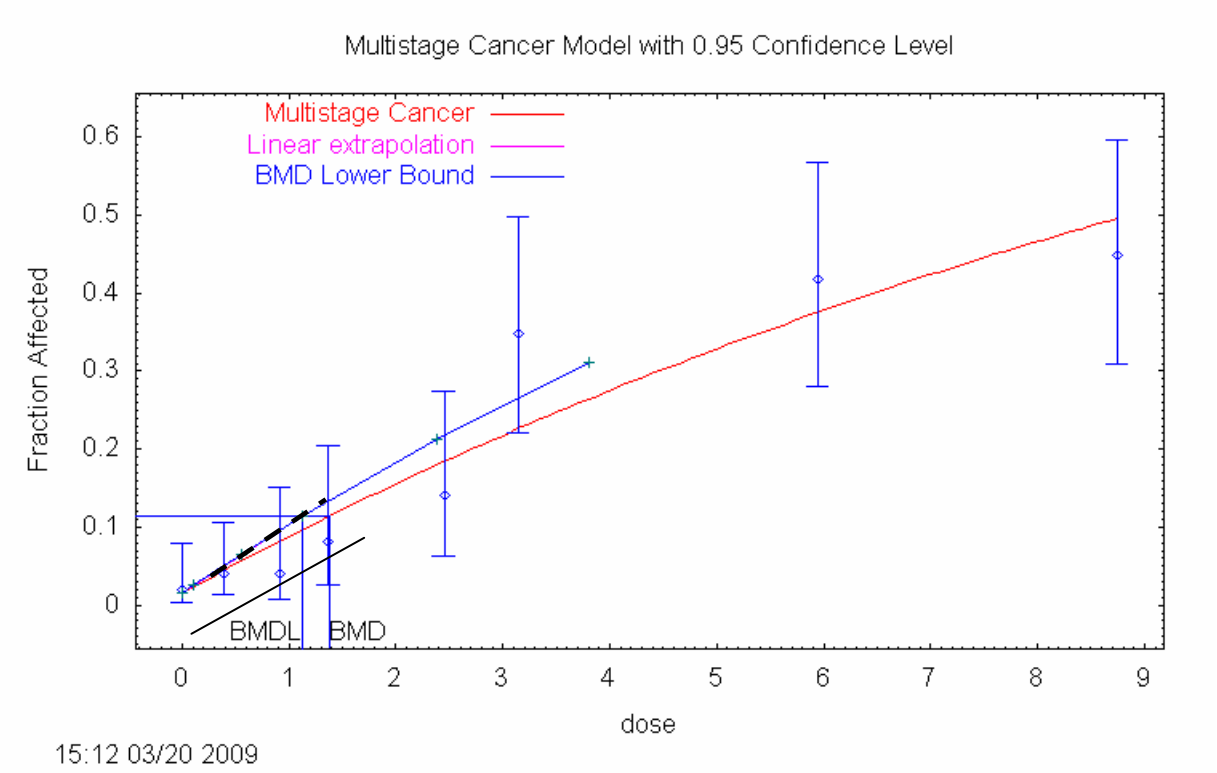


BMD (benchmark dose) – the dose corresponding to the 10% response rate after adjusting for response rate in controls

BMDL (benchmark dose-low) – the dose corresponding to the lower confidence bound on the 10% response rate after adjusting for response rate in controls

Fig. 7

Intestinal neoplasms in combined male and female mice benchmark dose modeling – Multistage cancer model

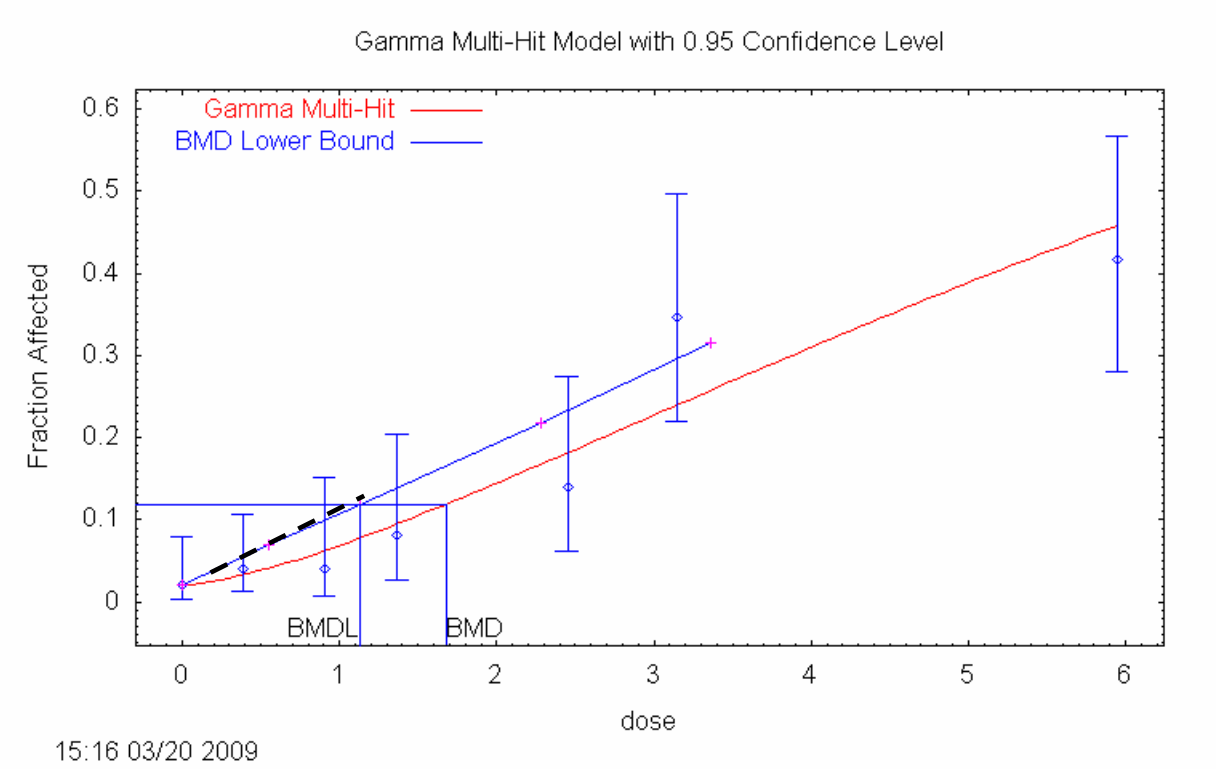


BMD (benchmark dose) – the dose corresponding to the 10% response rate after adjusting for response rate in controls

BMDL (benchmark dose-low) – the dose corresponding to the lower confidence bound on the 10% response rate after adjusting for response rate in controls

Fig. 8

Intestinal neoplasms in combined male and reduced female mice (female high-dose excluded) benchmark dose modeling – Gamma multi-hit model



BMD (benchmark dose) – the dose corresponding to the 10% response rate after adjusting for response rate in controls

BMDL (benchmark dose-low) – the dose corresponding to the lower confidence bound on the 10% response rate after adjusting for response rate in controls

Fig. A-1a

Concentration of Cr in female mouse kidney tissue at selected times in conjunction with drinking water exposure to sodium dichromate

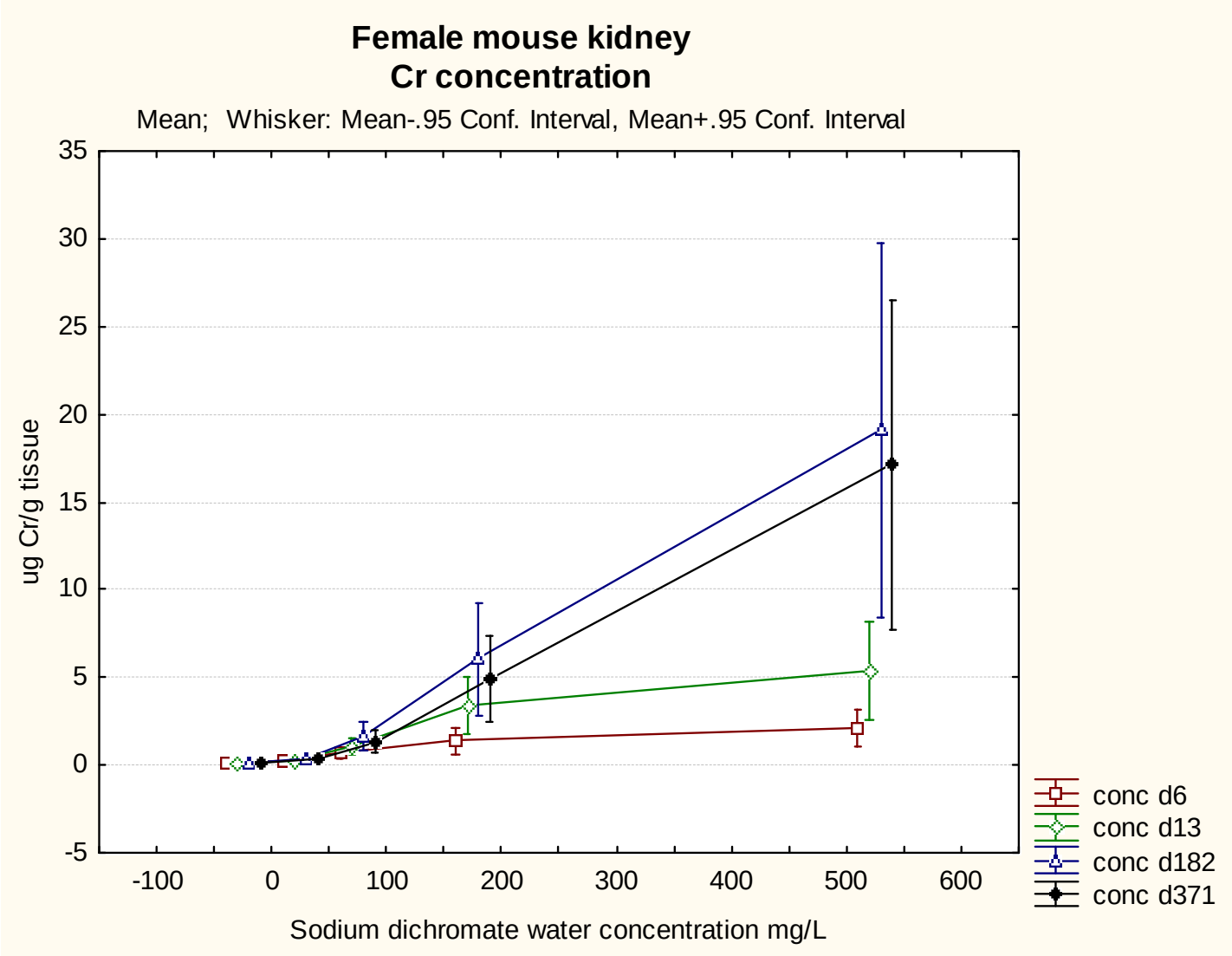


Fig. A-1b

Concentration of Cr in female mouse liver tissue at selected times in conjunction with drinking water exposure to sodium dichromate

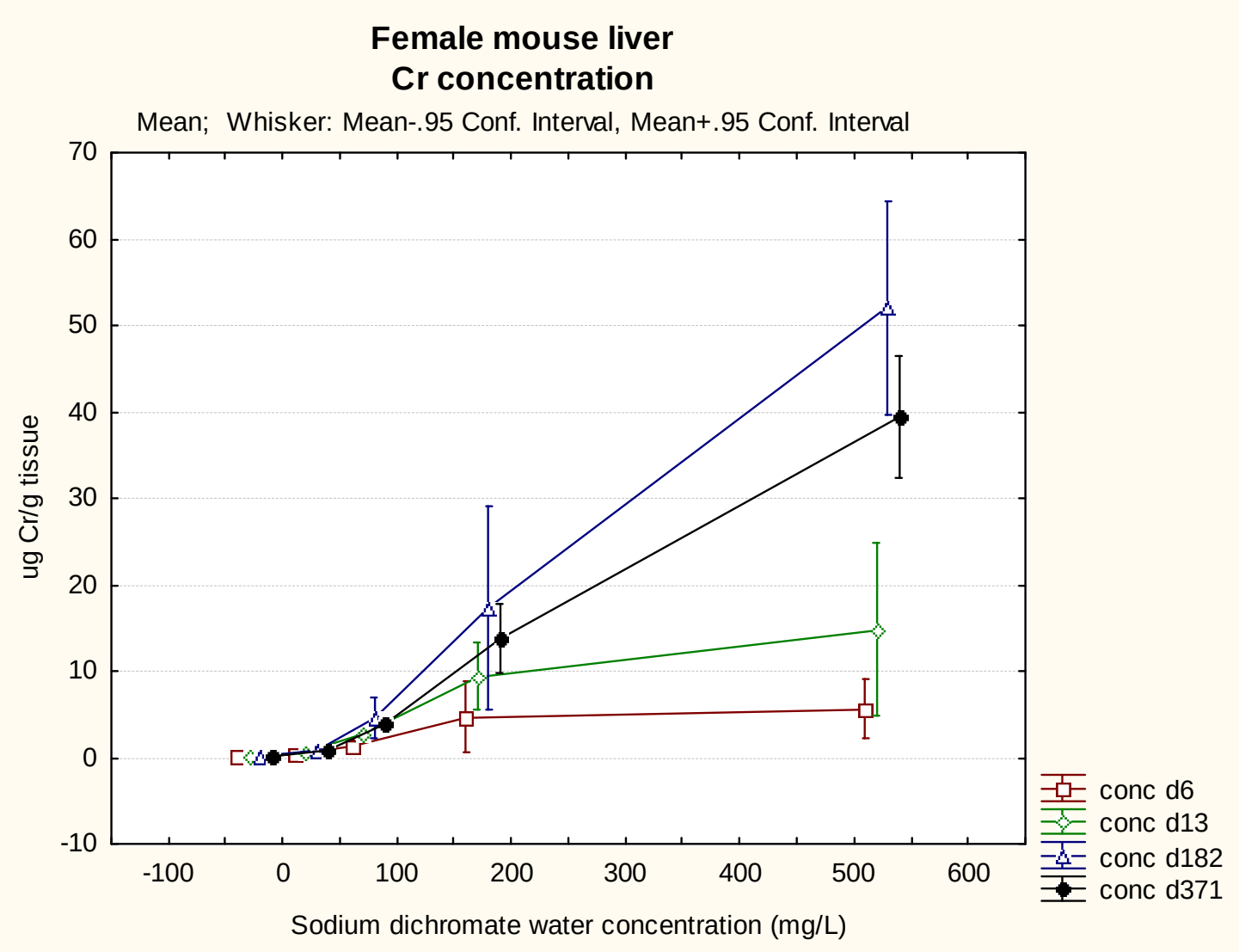


Fig. A-1c

Concentration of Cr in female mouse non-glandular stomach tissue at selected times in conjunction with drinking water exposure to sodium dichromate

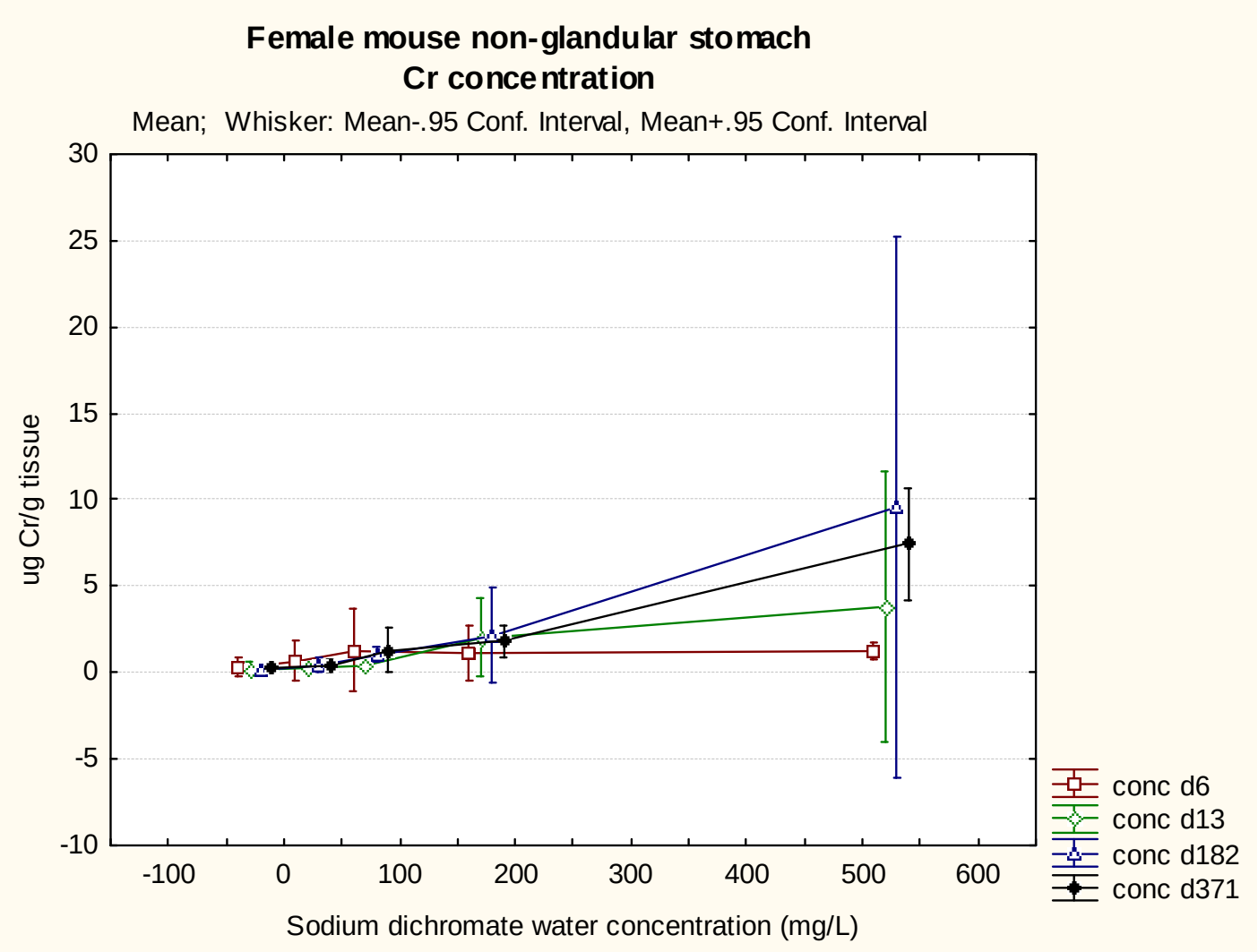


Fig. A-1d

Concentration of Cr in female mouse glandular stomach tissue at selected times in conjunction with drinking water exposure to sodium dichromate

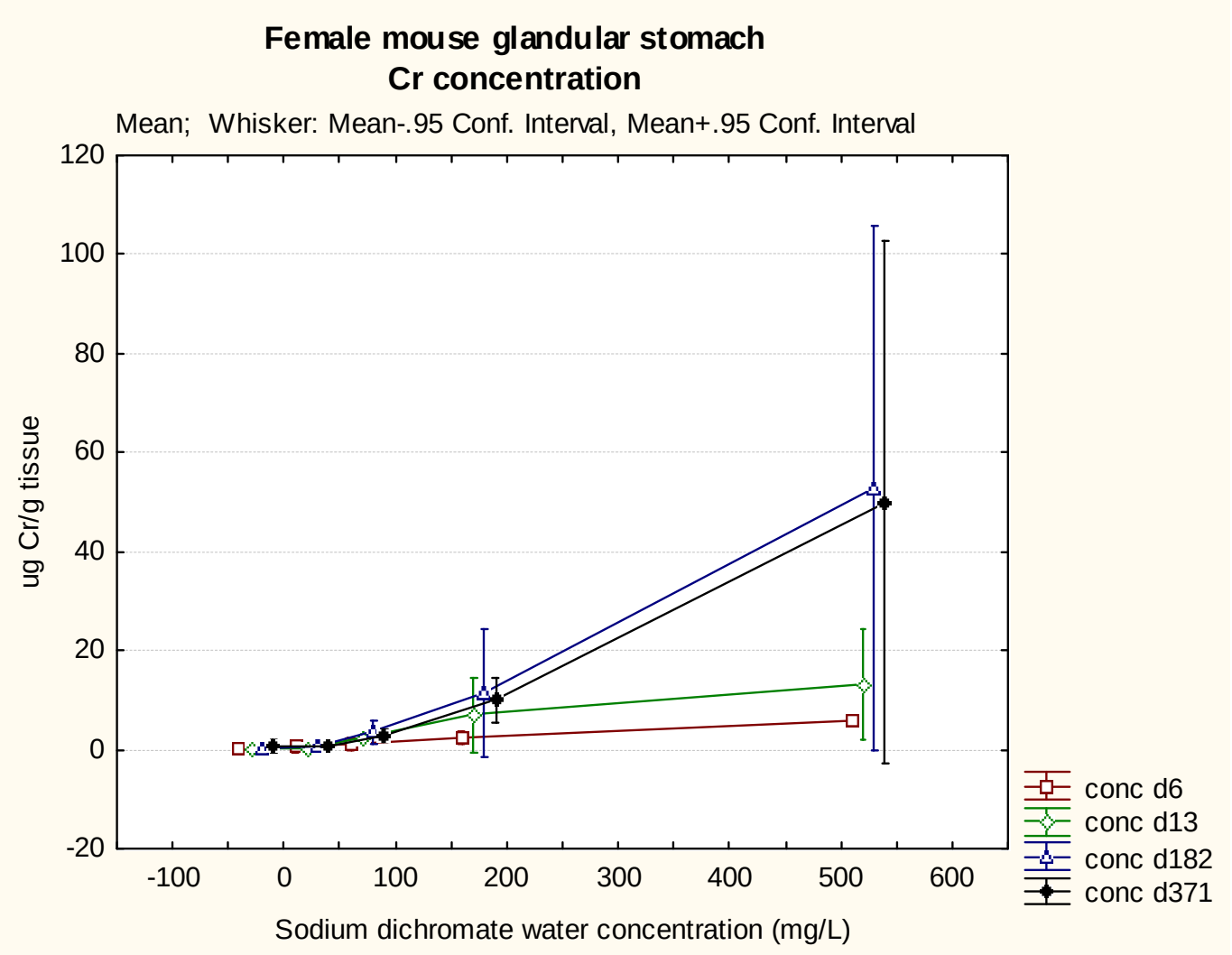


Fig. A-1e

Concentration of Cr in female mouse plasma at selected times in conjunction with drinking water exposure to sodium dichromate

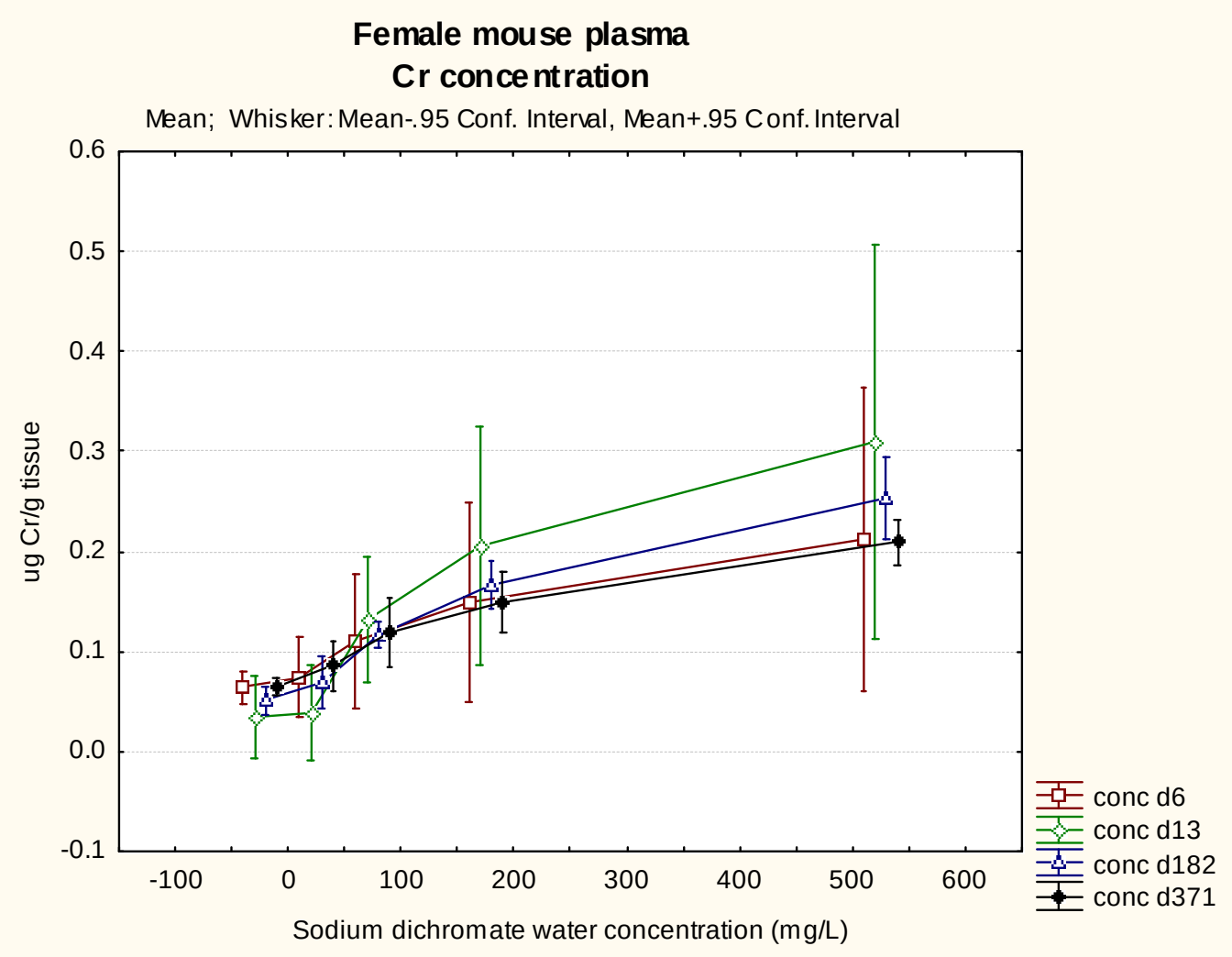


Fig. A-1f

Concentration of Cr in female mouse erythrocytes at selected times in conjunction with drinking water exposure to sodium dichromate

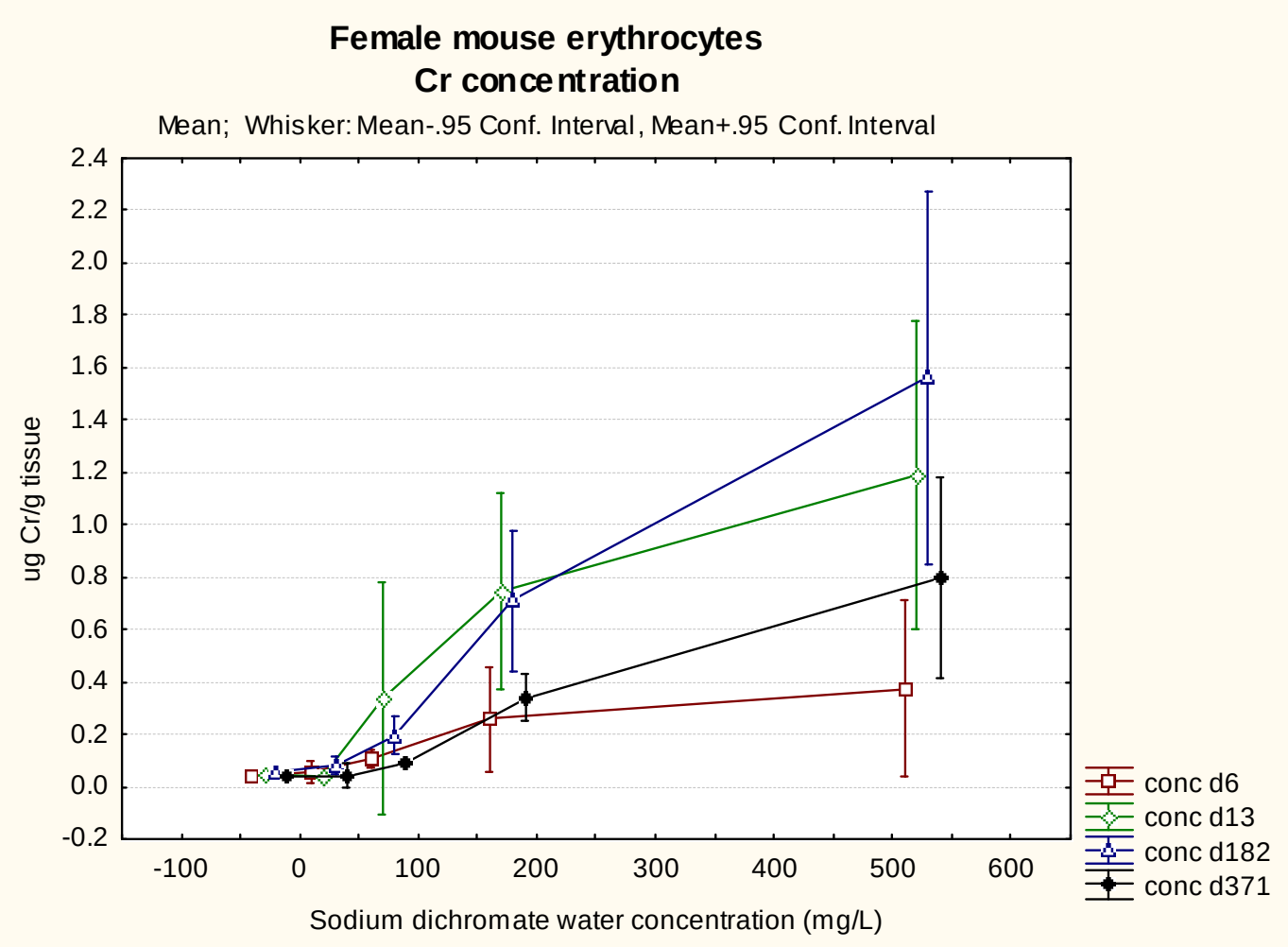


Fig. A-1g

Concentration of Cr in female mouse urine at selected times in conjunction with drinking water exposure to sodium dichromate

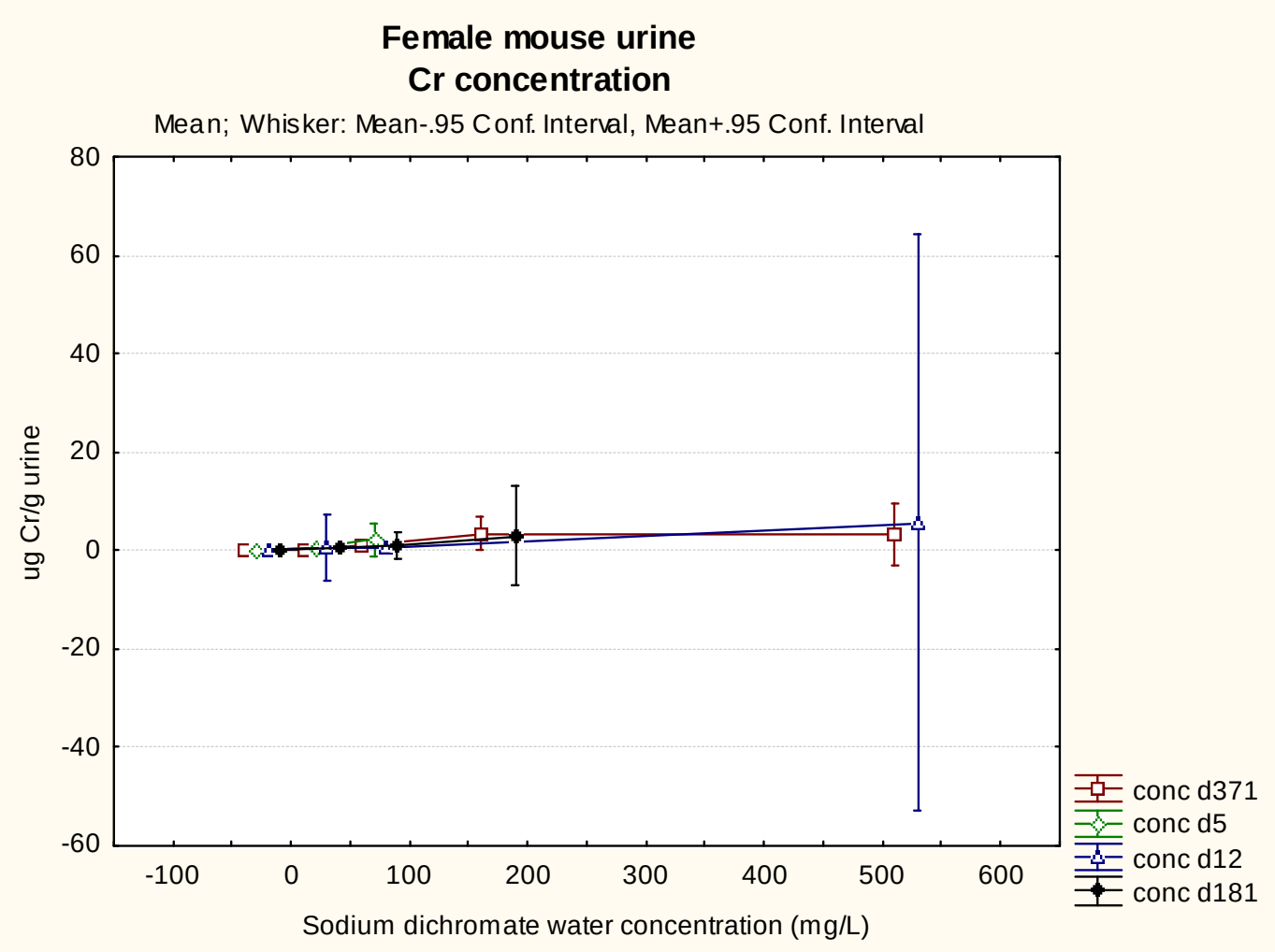


Fig. A-2

Concentration of Cr in male mouse blood and kidney after 21 days of exposure in conjunction with drinking water exposure to sodium dichromate