**Physiologically Based Pharmacokinetic Model for Humans Orally Exposed to Chromium**

Kirman C.R.1, Aylward L.L.2, Suh M. 3, Harris M.A.4, Thompson C.M.4, Haws L.C.5; Proctor D.M.3, Parker W.6, Lin S.6, Hays S.M.7

1Summit Toxicology,

29449 Pike Drive

Orange Village, OH 44022

216-544-8563

[ckirman@summittoxicology.com](mailto:ckirman@summittoxicology.com)

2Summit Toxicology, Falls Church, VA

3ToxStrategies, Rancho Santa Margarita, CA

4ToxStrategies, Katy, TX

5ToxStrategies, Austin, TX

6Duke University, Durham, NC

7Summit Toxicology, Lyons, CO

**Abstract.** A multi-compartment physiologically based pharmacokinetic (PBPK) model was developed to describe the behavior of Cr(III) and Cr(VI) in humans. Compartments were included for GI lumen, oral mucosa, stomach, small intestinal tissue (duodenum, jejunum, ileum), blood, liver, kidney, bone, and a combined compartment for remaining tissues. As chronic exposure to high concentrations of Cr(VI) in drinking water cause small intestinal cancer in mice, the toxicokinetics of Cr(VI) in the upper gastrointestinal tract in rodents and humans are important for assessing internal tissue dose and risk assessment. Fasted human stomach fluid was collected and e*x vivo* Cr(VI) reduction studies were conducted and used to characterize reduction of Cr(VI) in human stomach fluid as a mixed second-order, pH-dependent process. For model development, toxicokinetic data for total chromium in human tissues and excreta were identified from the published literature. Overall, the PBPK model provides a good description of chromium toxicokinetics and is consistent with the available total chromium data from Cr(III) and Cr(VI) exposures in typical humans (i.e., model predictions are within a factor of 3 for approximately 85% of available data). By accounting for key species differences, sources of saturable toxicokinetics, and sources of uncertainty and variation, the rodent and human PBPK models can provide a robust characterization of toxicokinetics in the target tissue of the small intestine allowing for improved health risk assessment of human populations exposed to environmentally-relevant concentrations.

**Keywords:** chromium, toxicokinetics, physiologically based model, gastrointestinal tract, human

**Abbreviations:**

CL = Lumen Cr Concentration

Cr = chromium

Cr(III) = trivalent chromium

Cr(VI) = hexavalent chromium

GI = gastrointestinal

KM = Michaelis-Menten constant

KRED = Rate constant for Cr(VI) reduction

L = Small intestines section length;

PBPK = physiologically based pharmacokinetic

PPI = proton pump inhibitor

RA = Relative Absorption

SDD = sodium dichromate dihydrate

SIDMS = speciated isotope dilution mass spectrometry

VM = Michaelis-Menten maximal rate

**1. Introduction**

Human exposures to low concentrations of hexavalent chromium, Cr(VI), are known to be widespread throughout the United States[1,2] and around the world[3,4]. Although the occurrence of high concentrations of Cr(VI) in environmental media is generally attributed to industrial releases, low concentrations of Cr(VI) in drinking water is prevalent from natural sources associated with Cr-enriched geology[1,3,5]. In addition to groundwater, low concentrations of Cr(VI) have been reported in urban household dust[6] and in some foods[7]. Because of this widespread exposure to Cr(VI), the potential for adverse health effects is of significant public health interest.

Chronic exposures to high concentrations of Cr(VI) in drinking water has recently been shown to increase the incidence of small intestinal tumors in mice, and oral cavity tumors in rats[8,9]. Although Cr(VI) is known to cause lung cancer in humans in specific industries and is known to induce respiratory tumors in animals following inhalation and intrabronchial administration[10], oral exposures were not previously thought to pose a cancer risk because most Cr(VI) is reduced to the trivalent form [Cr(III)] in the acidic environment of the stomach lumen[11,12]. In addition, Cr(VI)-exposed workers have not been shown to have an increased risk of gastrointestinal (GI) tract cancers[13]. Thus, the observation of GI tract tumors in rodents raises important questions regarding interspecies as well as high-to-low dose extrapolation used to estimate cancer risk associated with low-dose ingestion of Cr(VI) in humans.

Due to analytical limitations [i.e., difficulty in speciating Cr(III) and Cr(VI)], only total chromium was measured in tissues and excreta. Because measurements for total tissue chromium may not be the best internal dose measure for assessing cancer risk attributable to Cr(VI) exposures, a physiologically based pharmacokinetic (PBPK) model is needed to characterize reduction of Cr(VI) to Cr(III) prior to absorption and estimate internal doses for speciated chromium. Previous PBPK models for Cr(VI) and Cr(III) have been developed for rats and humans[14,15], but they do not include compartments and parameterization for the GI tract. More recently, a PBPK model has been developed for oral exposures to Cr(III) and Cr(VI) in rats and mice[16], which includes compartments for target tissues of interest in the GI tract (small intestines and oral cavity) and incorporates *ex vivo* data for the reduction of Cr(VI) in rat and mouse gastric contents[17].

The work presented here is part of multifaceted research effort to provide the data and tools needed to support human health risk assessment for Cr(VI). The study was designed using U.S. EPA risk assessment guidance[18] to address data gaps in the hypothesized mode of action (MOA) for Cr(VI) in mouse small intestines[19]. To date, these efforts have resulted in publications on the reduction of Cr(VI) in rodent gastric contents[17], analysis of toxicogenomic responses in rodent small intestines[20,21], and evaluation of biochemistry and histopathology in mouse and rat oral mucosa and small intestine[22,23]. In addition, a PBPK model has been developed for Cr in rodents[16]. The current study includes collection of *ex vivo* data on the rate and capacity of Cr(VI) reduction in fasted human gastric fluid. The goal of the human model developed here primarily to determine the rate and extent of absorption for Cr(III) and Cr(VI) from available human data sets, so that estimates of internal dose to the small intestine can be made. PBPK model-derived target tissue doses for the small intestines will be used to support quantitative risk assessment for oral exposures to Cr(VI), and provide a more scientifically supportable basis than default methods for interspecies and high-to-low-dose extrapolations.

**2. Material and Methods**

**2.1 Ex Vivo Studies of Cr(VI) Reduction Kinetics in Human Gastric Fluid**

We previously reported the kinetics of Cr(VI) reduction in rodent stomach contents[17]. It was determined that the reduction of Cr(VI) occurred by a mixed second-order model in which the rate of Cr(VI) reduction was dependent upon the concentration of Cr(VI) in the stomach fluid and the concentration of reducing agents present in the stomach contents. The experiments in rodent stomach contents were conducted at a range of Cr(VI) spiking concentrations and dilutions so as to assess reduction under conditions consistent with that of recent rodent 90-day and 2-year bioassays[9,22,23]. Rate constants (k) in mice and rats were 0.2 and 0.3 L mg-1 hr-1, respectively, and the reducing capacity of gastric contents was approximately 16 mg Cr(VI) reducing equivalents per L of stomach contents in both species[17].

Because De Flora et al.[24] determined the extent of Cr(VI) reduction at a single time point (60 minutes), their results are best used to characterize reduction capacity, rather than rate. De Flora et al.[24] found that the capacity of stomach fluid to reduce Cr(VI) was inversely related to pH (e.g., less reduction capacity at higher pH), but was also highest post-prandial compared to the fasted state (despite a higher pH). A strong pH dependence has been reported for the rate of reduction of Cr(VI) by glutathione[25], one of several potential reducing agents in the intestinal lumen. Thus, pH of the gastric fluid, and the presence of food (as source of reducing agents such as ascorbic acid) were considered to be important factors affecting Cr(VI) reduction in human stomach fluid.

*Ex vivo* studies using fasted human gastric fluids were conducted to assess the rate and capacity for Cr(VI) reduction. The gastric fluids were collected from 10 fasting preoperative cardiac patients at Duke University Hospital who were not on proton pump inhibitors (PPIs). Gastric fluid samples (1-10 mL) were collected from each patient, yielding a total gastric fluid volume of approximately 34 mL. To understand the effect of PPI use on gastric pH and Cr(VI) reduction, additional gastric fluid samples (2-3 mL) were collected from each of 5 patients on PPIs at Duke University Hospital, yielding a total gastric fluid volume of approximately 11 mL. Samples of all 10 patients, not on PPIs, were pooled by pH with a pool for sample at pH of ~1 (n= 3), a pool for pH ~2 (n=2) and a pool of pH ~4 (n= 5). The samples from 5 patients who were on PPIs were pooled separately and had a pH of ~7. The *ex vivo* approach to quantifying reduction kinetics of Cr(VI) in stomach fluids using speciated isotope dilution mass spectrometry (SIDMS) have been described previously[17]. The limit of detection in 10:1 diluted stomach fluid samples varied from sample to sample (≤5 ppb).

**2.2 Human PBPK Model Development**

Information from the published literature regarding the toxicokinetics of chromium in humans was reviewed to identify key data sets used in PBPK model development. These data include controlled exposures of human volunteers to Cr(III) and/or Cr(VI), and case reports of accidental or intentional poisonings with Cr(VI).

Although a PBPK model has been developed previously for chromium in humans[14], inspection of the model structure, parameterization, and predicted behaviors revealed that it would not be easily modified to reflect the key processes identified for assessing Cr toxicokinetics in GI compartments. For this reason a new conceptual and a new PBPK model were developed for human oral exposures to chromium. Code for the human PBPK was adapted from the rodent PBPK model[16] to describe the key toxicokinetic processes identified in the conceptual model. All PBPK modeling was performed in Advanced Continuous Simulation Language eXtreme (acslX) along with its interface for Excel (Aegis TG, version 3.0). Model parameter values were set based on: (1) data from the published literature; (2) by adjusting parameter values to obtain fits to the key data sets; and (3) professional judgment. The general approach to modeling the chromium data sets involved first defining model parameter values for Cr(III), then holding the parameters for Cr(III) toxicokinetics and for Cr(VI) reduction fixed, the Cr(VI) model parameters were adjusted to obtain fits to the key data sets identified for Cr(VI). Code was included in the model to track chromium mass-balance (see **Appendix A**), and all model simulations were checked to ensure mass-balance was maintained. The data sets of Finley et al.[26] were not used to estimate model parameters, but were instead held back to be used for model validation purposes. To help identify important model parameters, a sensitivity analysis was conducted for the human model by increasing individual model parameter values one at a time by 5% over their default values for a defined exposure simulation (exposure to 0.1 mg/kg spread over 5 equal exposure events per day) and noting the percent change in value predicted by the model for several internal dose measures.

**3. Theory**

**3.1 Key Data Sets**

Key data sets for understanding the toxicokinetics of Cr(III) and Cr(VI) in humans are listed in **Table 1** and described briefly below.

Several studies provide data for chromium in blood and urine levels in humans following oral exposures to Cr(III). Anderson et al.[27,28,29] examined serum and urinary chromium levels in a group of 76 volunteers (48 male, 28 female) exposed to 0.2 mg Cr/day as chromic chloride for up to 3 months. Mohamedshah et al.[30] examined chromium levels in plasma and urine from 6 lactating women supplemented with 0.4 mg/day chromium as chromic chloride for 3 days. Kerger et al.[31] measured blood and urine chromium levels in 4-5 volunteers receiving Cr(III) as chromic chloride or as Cr(VI) reduced to Cr(III) in orange juice. Volpe et al.[32] measured chromium in plasma, erythrocytes, and urine in 44 women administered 0.4 mg Cr/day as chromium picolinate for 12 weeks. Lukaski et al.[33] measured plasma and urine chromium levels in 83 women receiving 0.2 mg Cr/day as chromium picolinate for 12 weeks. Rubin et al.[34] assayed chromium levels in urine of 10 men receiving a single dose of 0.3 mg Cr as chromic chloride. Gargas et al.[35] measured urinary levels of chromium in 8 volunteers receiving 3 daily doses of 0.4 mg Cr as chromium picolinate. Overall, these data support a conclusion that the oral absorption of Cr(III) following short-term exposures is low (~0.1-2%), and that absorbed Cr is primarily excreted in the urine.

Several studies have been published that provide chromium data for blood, urine, and tissues following oral exposures to Cr(VI). Finley et al.[26] measured blood and urine levels of chromium in 5 volunteers exposed to water solutions containing 0.1-10 mg Cr/L as potassium chromate. Paustenbach et al.[36] (1996) measured blood and urine levels of chromium in a volunteer receiving drinking water containing 2 mg Cr/L as potassium dichromate for 17 days. Kerger et al.[31] measured chromium in blood and urine in 5 volunteers receiving a single dose of 5 mg Cr in water as potassium dichromate. Kerger et al.[31] demonstrated that bioavailability of Cr is greater and half-life longer upon Cr(VI) administration (bioavailability of 6.9%, and half-life of 39 hours) than administration of Cr(III) as chromium chloride (bioavailability of 0.13%, half-life of 10 hours) or as Cr(VI) reduced to Cr(III) in orange juice (bioavailability of 0.6%, and half-life of 17 hours). A number of case reports of Cr(VI) poisonings have reported blood, urine, and tissue levels (in fatal cases) of chromium[37,38,39,40,41,42]. The erythrocyte data from several studies[26,31,36] were not considered useful for modeling purposes due to concerns that erythrocytes were not sufficiently rinsed, resulting in artificially high concentrations due to cell membrane binding (i.e., not intracellular Cr) (Kerger, personal communication). Like the Cr(III) data, these data support a conclusion that the oral absorption of chromium [as a combination of Cr(III) and Cr(VI)] is higher than that of Cr(III), but still low, and that Cr that is absorbed is primarily excreted in the urine.

In reviewing toxicokinetic data for rodents[16], two important patterns were identified:

1. The ratio of chromium concentrations in erythrocytes:plasma serves as a potential biomarker for systemic Cr(VI) absorption. This ratio is generally 1 or less for Cr(III) exposures and low-dose exposures to Cr(VI), but increases above 1 for sufficiently high exposures to Cr(VI). At doses of Cr(VI) that exceed the GI tract capacity for reduction, Cr(VI) reaching portal plasma can enter portal erythrocytes. Following intracellular reduction to Cr(III), which is much less permeable to cell membranes, chromium becomes effectively “trapped” within erythrocytes.
2. There are clear species differences in the liver:kidney ratio of tissue chromium concentrations (ratio in mice generally greater than one; in rats, less than one).

These patterns appear to be relevant to humans as well. For example, the erythrocyte:plasma ratio in humans exposed to trivalent chromium for 12 weeks remain below a value one (approximately 0.3-0.6[32]), but ratios well above one (up to 26) can be calculated from data for human exposed to high doses of Cr(VI)[39,40,42]. With respect to liver:kidney concentration ratios, autopsy samples for background levels of chromium in human populations[43,44] and from case reports of high-dose Cr(VI) poisonings[37,38,40] report data that yield liver:kidney ratios greater than one (approximately 1.4-4.3). These data suggest that, across a broad range of exposures, the systemic distribution of chromium in humans appears more similar to that observed in mice than in rats.

**3.2 Conceptual Model**

A conceptual model was developed to describe the toxicokinetics of chromium in rats and mice (**Figure 1**[16]). The conceptual model describes the key processes affecting Cr(VI) starting from ingestion to excretion. Because the toxicokinetic processes are expected to be qualitatively similar across species, the conceptual model developed for rodents[16] is considered to be equally applicable to humans, and is summarized briefly below.

Cr(VI) reaching the stomach becomes mixed with saliva, gastric fluid, food, and water, and while in the lumen of the stomach and small intestines, is subject to three competing processes: (1) transit through the intestinal lumen sections; (2) reduction to Cr(III); and (3) uptake/absorption into GI tissue. The reduction of Cr(VI) has been described as a pH-dependent process[24,25]. With respect to pH, a longitudinal gradient is present within the lumen of the stomach and small intestines with stomach << duodenum < jejunum < ileum. Bicarbonate is excreted into the duodenum to neutralize the acidity of the chyme (semi-fluid, partially digested stomach contents) passing from the stomach to the small intestine. Assuming there is consistency in the availability of reducing agents, the rate of Cr(VI) reduction in the small intestines lumen is expected to decrease as chyme moves from the duodenum to the ileum consistent with the increasing pH of the GI lumen. However the pH of the duodenum is approximately 6 and that of the ileum is approximately 7[45], and the difference in reduction rates at these pH values is quite minimal. A significant change in pH occurs between the lumens of the stomach, with a pH of 1-3, and the duodenum with a pH of 6. This change in pH between the stomach and the duodenum is much greater in normal humans (from approximately 1.25 to 6) than it is in rodents (from approximately 4.5 to 5)[46,47].

Within the GI tissue, Cr(VI) is subject to further reduction, while both forms of chromium may be absorbed into portal plasma, or returned to the GI lumen with sloughed cells. Chromium absorption is expected to occur primarily within the small intestine. Based on rodent studies[16], the rate of uptake of chromium from the GI lumen is greatest in the duodenum, and lower in jejunum and ileum. The majority of chromium in the GI lumen remains unabsorbed and is excreted in feces.

Cr(VI) that reaches portal plasma is subject to several competing processes: (1) reduction to Cr(III); (2) uptake into erythrocytes (followed by intracellular reduction, resulting in an increased erythrocyte:plasma ratio as described in **Section 3.1**); and (3) transit to the liver. Based upon evaluation of rodent data[16] it is expected that, with the exception of very high exposures (i.e., fatal poisonings), essentially all chromium entering systemic plasma from the hepatic/portal system will have been reduced to Cr(III). The distribution of Cr(III) from plasma to tissues is largely determined by its binding to transport proteins[48,49,50], which may help to explain how plasma chromium levels remain relatively low despite much higher concentrations in mammalian tissues[8,51]. Transferrin, a protein is important for delivery of iron to tissues, possesses two binding sites, one of which has a high affinity for Cr(III) (i.e., Cr(III) will displace iron at neutral pH)[48], and therefore is expected to play a role in the delivery of Cr(III) from the GI tract to tissues. A low-molecular weight protein that is capable of tightly binding Cr(III) has been detected in a number of mammalian tissues[48,52,53,54], and may play a role in transport from tissues to kidney and its ultimate excretion in urine. Biliary excretion of chromium is expected to be negligible[15].

**4. Results**

**4.1 Ex Vivo Studies of Cr(VI) Reduction Kinetics in Human Gastric Fluid**

Similar to rodent gastric contents, the kinetics of Cr(VI) reduction in fasted human stomach fluid exhibited mixed 2nd order reaction kinetics. The reduction reaction of Cr(VI) in fasted stomach fluid samples was found to be inversely related to pH (highest rates of Cr(VI) reduction occurring at lower pH values). Representative curves are provided in **Figure 2a,b**. The 2nd order mixed model was fit to all reduction curves and the resulting reduction rate constants were found to vary as a function of pH (**Figure 2c**). The best-fit line to this data yielded a slope very close to -1. A slope of -1 provides an essentially equivalent fit to the data while preserving one degree of freedom. The latter form of the model was chosen for its chemical basis (consistent with pH being a logarithmic function):

*kred = 44.5\*exp(-pH) Eq.1*

The constant of 44.5 L/mg-hr is comparable to the values derived previously for rats (27 L/mg-hr) and mice (18 L/mg-hr)[16]. A range of estimates obtained for reducing equivalents (4 to 10 mg/L gastric fluid; mean = 7 mg/L) is consistent with the range of reducing equivalents (or reductive capacity) reported by De Flora et al.[24] in stomach fluid during the fasted state (< 10 mg/L). In De Flora et al.[24], peak reductive capacity was observed during the 1-4 h periods after each meal and the range of reducing equivalents was reported for the fed state to be approximately 30 mg/L. Fed stomach fluid samples could not be obtained for our *ex vivo* experiment; thus, data from De Flora et al.[24] for the fed state were used to parameterize the human PBPK model. Therefore, estimates of reductive capacity (or reducing equivalents – mg/L) in the fed and fasted state were approximately 30 mg/L and 7 mg/L, respectively. The second order rate constant was assumed to be the same in the fed and fasted condition.

**4.2 Human PBPK Model Development**

**4.2.1 Model Structure**

Because the conceptual model for chromium in humans is qualitatively the same as that for rodents, the PBPK model structure defined for rodents was adopted for the human model (**Figure 3**), and includes model compartments for gastrointestinal tract (stomach, duodenum, jejunum, and ileum) and systemic tissues (blood, liver, kidney, bone, and a lumped “other” tissue compartment). However, the following changes were made to adapt the model code for fitting human data sets: (1) code was added to allow for multiple bolus exposure events per day (up to 6/day) to accommodate the exposure regimens implemented by some human studies; (2) two model parameters (SFin, SFout) were added to permit scaling of systemic tissue uptake and release rate constants from mice; (3) because the human data were generally collected at doses much lower than those assessed in rodents, there is no need to treat absorption as a saturable process (i.e., separate parameters for Vmax and Km), and therefore absorption was modeled in humans as a first order process (i.e., a single model parameter equivalent to Vmax/K) and (4) the oral cavity compartment was removed from the model since there are no human data available for the oral cavity, the rapid transit rates in the oral lumen were contributing substantial stiffness (i.e., requiring much smaller time steps & longer simulation times) to the differential equations for the GI tract portion of the model, and since it does not appear that internal tissue dose is useful for explaining oral cavity tumor response across species (i.e., oral cavity tissue concentrations were higher in mice (nonresponsive for oral tumors) than rats (responsive for oral tumors)[16].

Based upon the *ex vivo* results described in Section 4.1, the rate constant for Cr(VI) reduction in the GI lumen was defined using Eq. 1 above, and the 2nd order rate of reduction is then calculated as:

*RRED = [Cr]\*kred\*RE Eq. 2*

Where,

RRED = Rate of Cr(VI) reduction (mg/hr);

[Cr] = Concentration of Cr(VI) in GI lumen (mg/L); and

RE = Amount of reducing equivalent in GI lumen (mg).

Cr(VI) reducing equivalents (RE) in gastrointestinal lumen were modeled as capacity limited, with consumption and regeneration of REs occurring as intake occurs and gastric contents are replenished as a mixture of food, water, gastric fluid, and saliva. The reduction of Cr(VI) in gastrointestinal tissues (stomach, duodenum, jejunum, and ileum) was modeled as a saturable process, assuming classic Michaelis-Menten kinetics based erythrocyte:plasma data that suggest that high doses of Cr(VI) can exceed the capacity of GI tissues (as well as lumen) for Cr(VI) reduction[16].

Absorption rates for Cr(III) and Cr(VI) from the GI lumen into tissues were modeled as first order processes for the dose range of interest for relevant human data sets (approximately 0.003 to 0.14 mg/kg). A longitudinal gradient for absorption in the small intestines was permitted in the model for both Cr(III) and Cr(VI), to allow for differences in absorption per unit length for the duodenum, jejunum, and ileum using the following equation:

*Absorption Rate (mg Cr/hr) = RA\*L\*KABS Eq. 3*

Where,

RA = Relative Absorption (relative to duodenum rate, unitless);

L = Section length (cm);

KABS = first order rate constant for absorption (mg/cm-hr)

Once absorbed, systemic Cr(III) was modeled as belonging to two general pools: (1) a distributional pool, which describes the distribution of Cr(III) from the GI tract to tissue via plasma; and (2) a storage/excretion pool, which describes the release of Cr(III) from tissues and its ultimate excretion in the urine. For all forms of chromium, distribution between plasma and systemic tissues was determined by "transfer efficiency" terms, which are multiplied by tissue blood flows:

*Tissue uptake (mg/hr) = SFin\*TE\*CBP\*QT Eq. 4*

Where,

SFin = Scaling factor applied to transfer efficiencies estimated for the mouse[16];

TE = transfer efficiency (unitless);

CBP = concentration of chromium in blood plasma (mg/L); and

QT = tissue blood flow rate (L/hr).

Similarly, release of Cr from tissues was calculated as follows:

*Tissue Release (mg/hr) = SFout\*TE\*CT\*QT Eq. 4*

Where,

SFout = Scaling factor applied to transfer efficiencies estimated for the mouse[16];

TE = transfer efficiency (unitless);

CT = concentration of chromium in systemic tissue (mg/L); and

QT = tissue blood flow rate (L/hr).

Transfer efficiency terms for systemic tissues were scaled from mouse parameter values since available human data (see **Section 3.1**) indicate that the liver:kidney Cr ratio in humans is greater than unity (similar to observations made for the mouse, but not for the rat). The structure of the chromium PBPK model is depicted in **Figure 3**, and the acslX code for the model is provided in **Appendix A**.

**4.2.2 Model Parameterization**

4.2.2.1 Physiological Parameters

Physiological parameters (body weight, tissue volumes, tissue blood flows) were obtained from the published literature[45,55] (**Tables 2** and **3**). GI transit rates, which apply to the transit of chromium and chromium reducing equivalents, were obtained from the published literature[44]. Transit times estimated for the small intestines were apportioned between the duodenum, jejunum, and ileum according to their relative lengths[44]. GI lumen pH and rates for food consumption, saliva production, and gastric fluid production were obtained from the published literature[44,47,56,57].

4.2.2.2 GI Lumen Cr(VI) Reduction

Model parameters for the reduction of Cr(VI) in the GI tract lumen, including pH-dependent rate constants and concentrations of reducing equivalents in stomach contents, are based on *ex vivo* data and modeling for the data described in **Section 4.1**. Based upon pH values of 2.5, 6, 6.5, and 7 for lumina of the stomach, duodenum, jejunum, and ileum lumen, Cr(VI) reduction rate constants of 3.7, 0.11, 0.067, and 0.041 L/mg-hr, respectively, for these model compartments were calculated using Equation 1. Model parameters for the reduction of Cr(VI) in GI tissues were defined as follows. The rate (V/K) of reduction in GI cells was assumed to be the same as estimated in rat erythrocytes (71 hr-1; see below). The value of Km for reduction in GI tissue was assumed to be equal to that estimated in rats and mice[16]. Model parameters for the reduction of Cr(VI) in systemic tissues are derived from limited available datasets. Reduction rates in plasma (0.66 hr-1) and erythrocytes (71 hr-1) are based on fits to *in vitro* rat data from Richelmi and Baldi[58], and were adopted for humans as well. Because pH is not expected to vary within systemic tissues, and because the availability of intracellular reducing equivalents are not expected to be rate limiting for low-dose exposures, the reduction value of 71 hr-1 was applied to all tissues, independent of pH and concentration.

All other model parameters were obtained by adjusting their values to obtain model predictions that fit available human data sets based upon visual and statistical optimization. Prior to modeling, tissue time-course concentrations for the key data sets (**Table 1**) were converted to added chromium, which was calculated as: (tissue chromium concentration in exposed individuals)-(tissue chromium concentration in non-exposed individuals). In most cases, study-specific information for background levels were used to calculate added chromium values; however, in cases where background levels were not clearly provided, information from other studies were used as indicated in **Table 1**. Studies that relied upon exposures to stable isotopes of chromium[30,34] did not require subtracting out background levels to determine added chromium.

4.2.2.3 Cr Absorption

The rate constant for absorption of Cr(III) in the small intestines (kabs3) was adjusted to match the total mass of chromium excreted in urine (i.e., the last time point for cumulative urinary excretion data). Values for kabs3 were adjusted separately for each data set, yielding a range of values (1.1-8.8E-05 L/hr-cm; mean=4.2E-05 L/hr-cm) (**Figures 4-11**). The range for kabs3 for data sets involving exposures to chromium chloride (1.1-8.8 L/hr-cm) was similar to that estimated for data sets involving exposures to chromium picolinate (2.0-8.3 L/hr-cm). The absorption of Cr(III) was greater when administered as Cr(VI) reduced in orange juice to Cr(III) compared to administration as CrCl3 [31], requiring an approximate 3-fold increase in the absorption rate constant (3.6 vs. 1.1 L/hr-cm) to achieve agreement with the data. The mechanism for this increase is not known, but is similar to the effect of orange juice on iron absorption, which has been attributed to ascorbic acid[59,60]. Difficulties were encountered in fitting the data from Lukaski et al.[33]. Specifically, adequate fits could be obtained to either the urine data or plasma data, but not both simultaneously, resulting in two estimates for Cr(III) absorption (**Figure 9**). Greater confidence is put in the absorption rate estimate based on plasma data since the result value (kabs3= 3.3E-05 L/cm-hr) falls within the range defined by other data sets, while that based on the urinary excretion data (kabs3 = 2.9E-04) falls well outside of this range.

Exposures to Cr(VI) result in absorption of chromium as both Cr(III) and Cr(VI). In simulating Cr(VI) data sets, model parameters for GI lumen reduction, Cr(III) absorption, and Cr(III) clearance were held constant (e.g., kabs3 was set equal to the mean value of 4.6E-05 L/hr-cm). Model parameter values for Cr(VI) were then adjusted to account for increased levels of chromium in measured plasma, erythrocytes, and urine. The rate constant for absorption of Cr(VI) in small intestines (kabs6) was adjusted to match the total mass of Cr excreted in the urine (i.e., last time point in cumulative urinary excretion plots). Values for kabs6 were set separately for each data set, ranging from 0.8-1.5E-04 (**Figures 12-15**).

Data are not available regarding the tissue concentrations in human small intestines following exposures to chromium. For this reason, tissue concentration data from pigs exposed to Cr(III) in the diet for 40 days were used as a surrogate (Wang et al., 2012). In this study, tissue levels of chromium measured in small intestines tissues corresponded to approximately 3% of total body burden. In modeling the Cr(III) data sets in humans, the model parameter for the release of Cr(III) from the small intestines to plasma (kout3) was adjusted to achieve a relative body burden of 3% for human small intestines. The model parameter for the release of Cr(VI) from small intestines to plasma in humans(kout6) was calculated from kout3 by assuming proportionality with rodent constants [kout6human = kout3human\*(kout6rodent/kout3 rodent), where the average ratio for rodent parameters was 3.6[16]].

4.2.2.4 Plasma Clearance

Datasets that provide time-course data (i.e., more than a single data point) for chromium in plasma following Cr(III) exposure[30,31] were used to estimate model parameters for systemic clearance of Cr from plasma. Similarly, data sets that provide time-course data for chromium in plasma following Cr(VI) exposure[31,36,42], also provide useful information on the systemic clearance of Cr(III) from plasma, since systemic levels largely reflect Cr(VI) that has been reduced to Cr(III) in the GI tract and portal system. Early time points for plasma were fit by adjusting model parameter values for systemic tissue uptake from the Cr distribution pool (SFin, kintrck), while late time points were fit by adjusting model parameter values for systemic tissue release (SFout) and renal clearance from the Cr excretion pool (kinccr). For the data set of Mohamedshah et al.[30], renal clearance for chromium from blood was best described using slightly lower values (kintcrk = 0.06; kinccr = 0.012). Because the subjects of this study were lactating women, the change in parameters may reflect changes in chromium clearance due to lactation. On the other hand, mode fits to the data from the individual studied by Paustenbach et al.[36] appeared to require a slightly faster clearance of Cr during the depuration phase (kinccr=0.06), than needed for the other data sets.

The rate constant for the uptake of Cr(III) from plasma into erythrocytes (krbcin3) was set by adjusting fits to the data set of Volpe et al.[32]. The value for the uptake of Cr(VI) into erythrocytes (krbcin6) and was set by adjusting model fits to the data of Goullé et al.[42] (**Figure 14**).

4.2.2.5 Urinary Excretion

Similarly, early time points for urine were fit by adjusting model parameters for renal clearance from the Cr distribution pool (kintcrk), while late time points were fit by adjusting model parameter values for renal clearance from the Cr excretion pool (kinccr). Parameter values for urinary excretion rate (kurcc) were adjusted to fit both early and late time points for urine, while ensuring that liver:kidney tissue predictions by the model remain above 1 during exposure period (consistent with the tissue concentration pattern identified in **Section 3.1**).

4.2.2.6 High-Dose Poisoning Studies

Attempts to fit the model to systemic tissue data from high-dose poisoning cases[37,38,40,41] were unsuccessful. For these data sets the model substantially overestimated plasma levels while substantially underestimating tissue levels of chromium (data not shown). The most likely explanation for this result is that the Cr(VI) doses in fatal case studies are well above the dose range evaluated for rodents (up to approximately 30 mg Cr(VI)/kg[16]). For example the doses estimated by Kolacinski et al.[39] and Loubieres et al.[40] were approximately 100 and 350 mg Cr(VI)/kg, respectively. At these high dose levels, Cr(VI) appears to overwhelm the reduction capacities of the GI lumen, GI tissue, and systemic tissues, resulting in systemic circulation of substantial levels of Cr(VI) that are not observed with low-dose exposures. At such high doses, the pattern of tissue concentrations conceptually consistent with a system-wide intracellular “trapping” of Cr in systemic tissues, similar to the smaller-scale “trapping” of Cr in portal erythrocytes at non-lethal doses (as discussed in **Section 3.1**). Because the model has not been parameterized for systemic distribution of Cr(VI), the model is not recommended for making predictions of internal dose when exposures exceed approximately 30 mg Cr(VI)/kg-day.

4.2.2.7 Model Validation and Performance  
For the purposes of model validation, model predictions were compared to the data set held back from model parameterization (i.e., Finley et al.[26]). Overall, the model predictions for plasma were in agreement with plasma data collected at 2 doses levels (5 and 10 mg Cr; **Figure 15a,b**), with model predictions falling within a factor of 3 for 8/9 measured data points. Unfortunately, the lower dose levels (0.1, 0.5 and 1.0 mg Cr) examined by Finley et al.[26] yielded plasma levels that were indistinguishable from pre-exposure levels, resulting in no useful data for added Cr to compare model predictions. Simulations for these low doses yielded plasma predictions are indistinguishable from pre-exposure level variation (1.96\*SD = 0.0012 mg/L; **Figure 15c**).

The PBPK model provides a reasonable description of the Cr(III) and Cr(VI) data sets for humans (**Figures 4-11**). Model predictions were found to be within a factor of 3 for approximately 84% of the measured data points across all Cr(III) data sets. Overall, the model predicts that fraction of ingested Cr(III) absorbed in these studies is low, ranging from approximately 0.002-0.015 (mean = 0.008). Model predictions were found to be within a factor of 3 or better for approximately 90% of the measured data points across Cr(VI) data sets. The predicted fraction of chromium absorbed [as a mixture of Cr(III) and Cr(VI)] is low (approximately 0.01) in the studies in which exposure was spread across multiple doses throughout the day[26,36], but is considerably higher (0.086) in one study in which exposure to Cr(VI) occurred as a single bolus dose[31]. The data of Goulle et al.[42] were best fit by the model using an estimated amount ingested of approximately 60 mg Cr(VI). This amount is larger than examined in controlled experiments discussed above, but is considerably smaller than estimated by the authors (approximately 3,000 mg), suggesting that the majority of Cr(VI) initially ingested by this individual was lost to vomiting. The PBPK model predicts that the fraction of Cr absorbed in this study was approximately 0.24, indicating that the fraction absorbed Cr increases as a function of Cr(VI) dose.

4.2.2.8 Sensitivity Analysis

A sensitivity analysis was conducted to identify model parameters that important to predicting internal dose measures of interest (**Table 4**). For measures of Cr(VI) flux (either amount leaving the stomach lumen normalized to SI tissue weight, or amount taken up by SI tissue normalized to SI tissue weight), both of which may be useful for evaluating point of contact effects in the small intestines, the most sensitive model parameters are GI lumen pH, GI lumen transit rates, GI lumen reducing equivalent concentration (and rate of regeneration), and GI lumen rate of reduction (**Table 4**). SI absorption rate for Cr(III), GI lumen pH, and duodenum length were the most sensitive model parameters affecting the fraction of absorbed chromium. For liver:kidney tissue concentration ratio, urinary excretion rate, systemic tissue uptake and release, and cardiac output were the most sensitive parameters. For erythrocyte:plasma tissue concentration ratio, GI lumen pH, GI lumen transit, plasma-erythrocyte transfer terms, and GI lumen reducing equivalents were identified as the most sensitive.

**5. Discussion/Conclusions**

*Ex vivo* reduction studies were conducted for Cr(VI) using human gastric contents to provide data required for modeling the toxicokinetics of chromium in the GI lumen. Based upon these data, Cr(VI) reduction was best described as a pH-dependent, 2nd order, capacity-limited process. These data were used to provide parameter estimates for the rate and capacity of Cr(VI) reduction in the human stomach. From this information and from information in the published literature, a PBPK model was developed to describe the toxicokinetics of chromium in humans. Overall, the model provides a good description of Cr(III) and Cr(VI) toxicokinetics in humans based upon available data, with model predictions falling within a factor of 3 or better for approximately 88% of the available data points (i.e., average of 84% for Cr(III) data sets, and 90% for Cr(VI) data sets). Furthermore, the PBPK model predictions are consistent with the key patterns (liver:kidney and erythrocyte:plasma ratios discussed in **Section 3.1**) identified for chromium. Model predictions for liver:kidney ratios were approximately 2 for the exposure scenarios evaluated, compared to the range of 1.4-4.3 identified from the published literature[37,38,40,43,44]. Similarly predictions for the erythrocyte:plasma ratio are less than 1 for Cr(III) exposures, which is consistent with the observed data of Volpe et al.[32]; while predicted ratios greater than 1 are consistent with observed data for high-dose exposures to Cr(VI)[39,40,42].

Several of the datasets used to fit the model parameters included data for multiple individuals. The approach of O’Flaherty et al.[14] was to fit their PBPK model parameters to the data for each individual separately, principally by varying the rate of Cr(VI) and Cr(III) absorption across individuals. The authors had noted another approach would have been to alter their (simplistic) rate of reduction for achieving fits to total Cr following Cr(VI) dosing. The approach we used was to average the data across individuals within a study as a function of added Cr and to use one set of model parameters to fit the entire study. Fitting data for individuals will provide greater insight on individual variability; however, the source of the variability (differences in absorption rate constant, concentration of reducing equivalents, pH of GI contents, rate of stomach emptying, etc.) will be indistinguishable. Therefore, the approach we have used for fitting the model to the average of the study participants provides estimates of average model parameter values. Assessing sources and ranges of variation in model parameters will be relevant for a risk assessment using this model.

The human PBPK model for chromium shares nearly the same structure as that developed for rats and mice[16]. However, there are some notable differences between rodents and humans. First, the rodent forestomach has no human tissue counterpart[61,62]. Because forestomach and glandular stomach were treated as a lumped compartment in the rodent PBPK model, there was no need to alter the model structure for humans. Second, there are species differences in the absorptive surface area of the small intestines[63,64], which may be an important factor for point of contact effects. Third, although data from high-dose rodent studies required inclusion of a saturable absorption algorithm for both Cr(III) and Cr(VI)[16], this was not required to fit the human data sets since the Cr(III) data sets were collected at low doses (i.e., below any potential saturation), and the Cr(VI) data sets were either collected at low doses repeated exposures or for high-dose acute exposures (i.e., too short to produce treatment-related effects on cell turnover that might impact absorption).

An additional important difference between rodents and humans pertains to variation. Factors such as gastric lumen pH, gastric lumen transit time, gastric lumen volumes, and gastric fluid production, are much more variable in humans than in rodents. As herbivores with a forestomach to store food and under *ad libitum* feeding conditions, mice and rats have relatively consistent stomach conditions with low variability in stomach pH, gastric emptying time, and relatively constant gastric acid production[17,46,57]. In humans, gastric reduction of Cr(VI) is dependent on the presence of food and pH[24]. Reduction capacity increases substantially in fed conditions because upon anticipation of eating and distention of the stomach, significant gastric acid, protein and enzyme production occurs. De Flora et al.[24] measured the capacity of the stomach to reduce Cr(VI) in a normal individual and 16 individuals who were hospitalized for duodenal ulcer and cholecystectomy. Circadian changes in Cr(VI) reduction capacity and pH were presented (graphically) for five individuals, including two under treatment with an agonist of histamine H2-receptor (famotidine). Although food itself contributes additional reducing agents, some increase in Cr(VI) reductive capacity in is likely related to acid and enzyme secretion (e.g., reductases that may regenerate reducing equivalents) that occurs with ingestion of a meal. Basal gastric acid production between meals in humans is relatively low, ~2-5 mEq/hr, but reaches peak rates of 18 to 23 mEq/hr upon food consumption[47]. Gastric pH is more highly variable in humans than in rodents, in fasting conditions normal gastric pH ranges from approximately 1 to 3 in humans, and in fed conditions pH increases to approximately 4-5[47,56,57]. In rodents, gastric pH differs only slightly between fed and fasted states (approximately 3 and 4, respectively)[46]. It is well recognized that reduction of Cr(VI) to Cr(III) is more rapid at low pH, in the presence of reducing agents, in the human stomach as well as in other environments[24,25]. Gastric lumen pH has been shown to vary with age[65,66,67,68], and also varies between individuals including potentially sensitive subpopulations (e.g., PPI users). In addition to being highly variable in humans, many of these parameters (lumen pH, gastric emptying time, lumen reducing equivalents) were identified as having a large effect on model predictions for the flux of Cr(VI) leaving the stomach and taken up by SI tissue (**Table 4**). For this reason, characterization of these important sources of human variation will serve as an important component of a PBPK-based human health risk assessment for chromium.

There are sources of uncertainty in this model, many of which have been discussed previously for the rodent PBPK model[16]. The rate of Cr(VI) reduction in human gastric contents estimated is based upon samples from fasted individuals. Although changes in gastric reduction capacity due to the presence of food were addressed using the data of DeFlora et al.[24], data are not available to estimate a different rate of reduction for individuals in a fed state, and therefore all human modeling has been performed using fasted state reduction rates. As noted for the rodent model, no data are available for describing intracellular reduction of Cr(VI) in the small intestines in either rodents or humans, requiring the use of surrogate data to characterize this reaction (i.e., reduction of Cr(VI) in rat erythrocytes). In addition, a key source of uncertainty in the human PBPK model is associated with the fact that the data available for chromium in exposed humans are primarily limited to plasma, erythrocytes, and urine. For this reason, consideration was given to combining all systemic tissues into a single compartment. However, separate systemic model compartments were maintained for the sake of consistency with the rodent PBPK model structure[16] and the previously published model[14]. In addition, separate model compartments permit a comparison between model predictions for liver:kidney data, for which limited data are available in humans following low-level exposures to Cr(III)[43,44] and fatal poisonings with Cr(VI)[37,38,40]. Another source of uncertainty pertains to the relative timing of Cr(VI) exposure events compared to the state of the gastrointestinal tract (i.e., fed or fasted states). For the sake of simplicity in fitting available human data sets, for which information regarding relative timing of exposure and GI state are lacking, constant daily average values were adopted for model parameters that are known to vary between fed and fasted states (lumen pH, gastric emptying, Cr(VI) reduction capacity). However, in applying the model to human health risk assessment (in preparation), more careful consideration will need to be given to the impact of these factors on tissue dosimetry and their implications on risk.

The application of the rodent and human PBPK models to human health risk assessment will require a careful consideration of the mode of action for the tumorigenic effects. For tumors in the mouse small intestines, potential candidate dose measures include those for Cr(VI) concentration (e.g., in the lumen or tissue of the small intestines) or Cr(VI) flux (e.g., Cr(VI) leaving the stomach lumen or entering into small intestines tissue). Selection of an appropriate dose measure will also need to include a consideration of the confidence in the PBPK model predictions. For example, although there is reasonable confidence in model predictions for small intestines tissue concentrations of Cr(VI) in mice because they are grounded by observed measurements made for total chromium[16], no such data are available for Cr in human small intestines. For this reason, human model predictions for Cr concentrations in small intestinal tissues cannot be validated. However, the flux of Cr(VI), transited from the lumen of the stomach to the lumen of the small intestines is primarily dependent upon our understanding of competing rates for GI transit and gastric Cr(VI) reduction, both of which have been well characterized in humans. Similarly, because the small intestines serve as the primary location for Cr absorption (based upon relative surface areas of GI segments), model predictions for flux of chromium into SI tissue, normalized to SI tissue volume or absorptive surface area, is sensitive to the levels of chromium reaching blood and urine, for which there are direct measurements in humans. Unfortunately, a similar approach cannot be made for the oral cavity, which lacks measured data in humans, and is expected to be a negligible contributor to systemic chromium levels such that estimates of flux are not practical.

PBPK modeling in human health risk assessment for chromium will permit evaluation of the uncertainty and variability in both the rodent and human model predictions for the dose measure(s) selected. By accounting for key species differences, sources of saturable toxicokinetics, and sources of uncertainty and variation, the rodent PBPK model[16] and human PBPK model presented here should provide risk managers with a more robust characterization of interspecies variability and improved extrapolation of rodent bioassay results to environmentally-relevant exposures in human populations.

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**Notes**

The DUHS IRB has determined that protocol ID: Pro00028884, for the collection of human stomach fluids, meets the definition of research not involving human subjects as described in 45 CFR 46.102(f), 21 CFR 56.102(e) and 21 CFR 812.3(p) and satisfies the Privacy Rule as described in 45 CFR 164.514.

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**Figure Legends:**

Fig. 1. Competing Toxicokinetic Processes for Chromium in the Gastrointestinal Tract: R1 = gastrointestinal transit; R2 = Cr6 reduction; R3 = Cr transport to epithelium; R4 = Cr absorption into blood; R5 = Cr transit in portal plasma to the liver; R6 = Cr in sloughed cells. Processes are depicted for a single section of the GI tract, but are applicable to model compartments for the stomach, duodenum, jejunum, and ileum in sequence.

Fig. 2. Ex Vivo Reduction of Cr(VI) in Human Stomach Fluid Collected From Preoperative Cardiac Patients Not On PPIs: (A) Time-course data for Cr(VI) spike concentration of 1 ppm, gastric fluid dilution of 10:1, pH=1.3 (n=10); (B) Time-course data for Cr(VI) spike concentration of 0.1 ppm, gastric fluid dilution of 10:1, pH=4; and (C) Relationship between pH and Cr(VI) reduction rate constant. diamonds = data points, lines = reduction model predictions

Fig. 3. PBPK Model Structure. RBC = red blood cells; S = stomach; D = duodenum; J = jejunum; I = ileum; LI = large intestines. All compartments can

contain Cr(VI), light shaded arrows and compartments depict Cr(III) in the

distribution pool; dark shaded arrows and compartment depict Cr(VI) in the

storage/excretion pool; intermediate shaded compartments (plasma) contain both

Cr(III) pools.

Fig. 4. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) for Humans Exposed to 0.4 mg Cr(III)/day (as chromium chloride) for Three Days[30]. diamonds = data points, error bars = standard deviation, solid line = model predictions (required slower clearance from plasma; kintcr=0.06, kinccr=0.012)

Fig. 5. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) for Humans Exposed to 0.2 mg Cr(III)/day (as chromium chloride) for up to Three Months[27,28,29]. diamonds = data points, error bars = standard deviation, solid line = model predictions

Fig. 6. Model Predictions for Cumulative Urinary Excretion of Chromium for Humans Exposed to a Single Dose of 0.3 mg Cr(III) (as chromium chloride)[34]. diamonds = data points, error bars = standard deviation, solid line = model predictions

Fig. 7. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) for Humans Exposed to a Single Dose of 5 mg Cr(III) (as chromium chloride)[31]. diamonds = mean (n=5), error bars = standard deviation, solid lines = model predictions

Fig. 8. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) for Humans Exposed to a Single Dose of 5 mg Cr(III) (as Cr(VI) reduced in orange juice)[31]. diamonds = mean (n=5), error bars = standard deviation, solid lines = model predictions

Fig. 9. Model Predictions for Chromium in Plasma and Cumulative Urinary Excretion for Humans Exposed to 0.2 mg Cr(III)/day (as chromium picolinate) for Twelve Weeks[33]. Simultaneous fits to both plasma and urine data were not possible, therefore absorption adjusted to provide model fits to plasma data (solid lines; kabs3=3.3E-05 L/hr-cm) and urinary excretion data (dashed lines; kabs3=2.9E-04).

Fig. 10. Model Predictions for Chromium in Plasma, Erythrocytes (A), and Cumulative Urinary Excretion (B) in Humans Exposed to 0.4 mg Cr(III)/day (as chromium picolinate) for Twelve Weeks[32]. diamonds and squares = data, error bars = standard deviation, solid and dashed lines = model predictions

Fig. 11. Model Predictions for Cumulative Urinary Excretion of Chromium in Humans Exposed to 0.4 mg Cr(III)/day (as chromium picolinate) for Three Days[35]. diamonds = mean (n=8), error bars = standard deviation, solid lines = model predictions

Fig. 12. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) a Single Human volunteer Exposed to 4 mg Cr(VI)/day (as dichromate) for Seventeen Days[36]. diamonds = data points, error bars = standard deviation, solid line = model predictions (required a faster plasma clearance, kincc=0.06)

Fig. 13. Model Predictions for Chromium in Plasma (A) and Cumulative Urinary Excretion (B) in Humans Exposed to a Single Dose of 5 mg Cr(VI)/day (as dichromate)[31]. diamonds = mean (n=5), error bars = standard deviation, solid lines = model predictions

Fig. 14. Model Predictions for Chromium in Plasma, Erythrocytes (A), and Cumulative Urinary Excretion (B) in an Individual Exposed to a Single Dose of Cr(VI) (as chromate)[42]. diamonds = data, error bars = standard deviation, solid lines = model predictions

Fig. 15. Model Predictions for Chromium in Plasma in Humans Exposed to a Single Dose of 10 mg (A), 5 mg (B), or 0.1-1 mg (C) of Cr(VI) (as chromate)[26]. diamonds = data, error bars = standard deviation, solid lines = model predictions, dashed line = upper bound estimate of background Cr levels (calculated as pre-exposure SD\*1.96)