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Physiologically based pharmacokinetic model for rats and mice orally exposed to chromium

C.R. Kirman [a,*], S.M. Hays [b], L.L. Aylward [c], M. Suh [d], M.A. Harris [e], C.M. Thompson [e], L.C. Haws [f], D.M. Proctor [d]

[a] Summit Toxicology, 29449 Pike Drive, Orange Village, OH 44022, USA
[b] Summit Toxicology, Lyons, CO, USA
[c] Summit Toxicology, Falls Church, VA, USA
[d] ToxStrategies, Rancho Santa Margarita, CA, USA
[e] ToxStrategies, Katy, TX, USA
[f] ToxStrategies, Austin, TX, USA

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ABSTRACT

A multi-compartment physiologically based pharmacokinetic (PBPK) model was developed to describe the behavior of Cr(III) and Cr(VI) in rats and mice following long-term oral exposure. Model compartments were included for GI lumen, oral mucosa, forestomach/stomach, small intestinal mucosa (duodenum, jejunum, ileum), blood, liver, kidney, bone, and a combined compartment for remaining tissues. Data from *ex vivo* Cr(VI) reduction studies were used to characterize reduction of Cr(VI) in fed rodent stomach fluid as a second-order, pH-dependent process. For model development, tissue time-course data for total chromium were collected from rats and mice exposed to Cr(VI) in drinking water for 90 days at six concentrations ranging from 0.1 to 180 mg Cr(VI)/L. These data were used to supplement the tissue time-course data collected in other studies with oral administration of Cr(III) and Cr(VI), including that from recent NTP chronic bioassays. Clear species differences were identified for chromium delivery to the target tissue (small intestines), with higher concentrations achieved in mice than in rats, consistent with small intestinal tumor formation, which was observed upon chronic exposures in mice but not in rats. Erythrocyte:plasma chromium ratios suggest that Cr(VI) entered portal circulation at drinking water concentrations equal to and greater than 60 mg/L in rodents. Species differences are described for distribution of chromium to the liver and kidney, with liver:kidney ratios higher in mice than in rats. Overall, the PBPK model provides a good description of chromium toxicokinetics, with model predictions for tissue chromium within a factor of 3 for greater than 80% of measurements evaluated. The tissue data and PBPK model predictions indicate a concentration gradient in the small intestines (duodenum > jejunum > ileum), which will be useful for assessing the tumor response gradient observed in mouse small intestines in terms of target tissue dose. The rodent PBPK model presented here, when used in conjunction with a human PBPK model for Cr(VI), should provide a more robust characterization of species differences in toxicokinetic factors for assessing the potential risks associated with low-dose exposures of Cr(VI) in human populations.

1. Introduction

Hexavalent chromium [Cr(VI)] has recently been shown to cause small intestinal tumors in mice, and oral cavity tumors in rats following chronic exposure to high concentrations of Cr(VI) in drinking water [1,2]. Although Cr(VI) is known to cause lung cancer due to occupational exposures in certain industries, and also induces respiratory tumors in animals following inhalation and intrabronchial administration [3], oral exposure to Cr(VI) was not thought to pose a cancer hazard previously because Cr(VI) is largely detoxified by reduction to trivalent chromium [Cr(III)] in the acidic reducing conditions of the stomach [4,5]. Thus, observation of gastrointestinal (GI) tract tumors in rodents exposed to very high concentrations of Cr(VI) in drinking water raises several important questions regarding the toxicokinetics of ingested

Abbreviations: AUC, area under the curve; CL, lumen Cr concentration; Cr, chromium; Cr(III), trivalent chromium; Cr(VI), hexavalent chromium; GI, gastrointestinal; KM, Michaelis–Menten constant; L, small intestines section length; PBPK, physiologically based pharmacokinetic model; RA, relative absorption; SDD, sodium dichromate dihydrate; VM, Michaelis–Menten maximal rate.

* Corresponding author. Tel.: +1 216 544 8563.
E-mail address: ckirman@summittoxicology.com (C.R. Kirman).

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Cr(VI). These questions include whether the reducing capacity of the stomach was overwhelmed by the high concentration exposures and how the toxicokinetics of Cr(VI) differ between humans and rodents [6–8]. Further, species differences in toxicokinetics may inform the mode of action (MOA) for carcinogenesis, and quantitative descriptions of the toxicokinetics may be used in PBPK modeling to refine extrapolation of target tissue dose between species and over the range of doses of interest for risk assessment [6].

Human exposure to low concentrations of Cr(VI) is now known to be widespread. Cr(VI) occurs at relatively low concentrations in drinking water throughout the United States [12,24], and in other locations around the world [9,10]. Although the occurrence of high concentrations of Cr(VI) in environmental media is typically due to industrial pollution, most low level Cr(VI) in drinking water is believed to occur from natural sources associated with Cr-enriched geology [9,11,12]. Cr(VI) has also been reported at low levels in urban household dust [7] and in some foods [13,14]. For these reasons, the cancer risk assessment of ingested Cr(VI) at environmentally-relevant exposures is of significant public health interest.

The dose–response for small intestinal tumors in the NTP bioassay for mice has been the basis of several health risk assessments of oral Cr(VI) exposure in humans [7,15,16]. In these assessments, however, default procedures for extrapolating between species (body weight scaling of dose to the ¾ power) and across dose (assumptions of linearity) were applied because rodent- and human-specific PBPK models that include parameterization of the gastrointestinal (GI) were unavailable. In these assessments, the tumor incidences for mouse duodenum, jejunum, and ileum were summed to estimate cancer potency based on administered dose without consideration of the longitudinal gradient in hyperplasia/tumor response, and likely differences in tissue dose by segment. Specifically, the strongest response was observed in mouse duodenum, a weaker response was observed in mouse jejunum, and a response was generally absent in mouse ileum, consistent with a gradient for portal of entry effect with decreasing target tissue dose with distance from the stomach.

Earlier PBPK models for Cr(VI) and Cr(III) have been developed for rats and humans [17,18], but they do not include parameterization of the GI tract. While these models potentially provide a valuable starting point for estimating kinetics of Cr(VI) and Cr(III), they were parameterized with very little oral dosing data. In fact, many kinetic parameters in the model were estimated using PK data from studies that utilized a single intra-tracheal administration of Cr(VI). Until the NTP cancer studies, the PK data following chronic oral exposures to Cr(VI) was fairly limited, particularly in mice.

The work presented here is part of multifaceted research effort to provide the data and tools needed to support Cr(VI) risk assessment. The study was designed using US EPA risk assessment guidance [22] and to address data gaps in the hypothesized mode of action (MOA) for Cr(VI) in mouse small intestines [6]. To date, these efforts have resulted in publications on the reduction of Cr(VI) in rodent gastric contents [21], analysis of toxicogenomic responses in rodent small intestines [70,71], and evaluation of biochemistry and histopathology in mouse and rat oral mucosa and small intestine [19,20]. The current study incorporates *ex vivo* data collected for the rate and capacity of Cr(VI) reduction in fasted rodent gastric contents [21]. In addition, a PBPK model is under development for Cr(III) and Cr(VI) in humans based upon the ex vivo reduction data and toxicokinetic data from the published literature (in prep). The model will be used to support quantitative risk assessment for oral exposures to Cr(VI) by: (1) improving extrapolations across species (i.e., from rodents to humans); (2) improving extrapolations from the high-dose exposures associated with tumors in rats and mice to low-dose environmentally-relevant exposures; (3) characterizing dose gradients in the small intestines that may help explain the response gradient for tumor formation in mice; and (4) characterizing target tissue doses in term of speciated forms of chromium [Cr(III) or Cr(VI)] rather than relying upon measurements of total chromium in tissues or upon administered dose. PBPK modeling is the preferred method for inter-species extrapolation because it offers a more robust approach as compared to default procedures such as allometric scaling (e.g., body weight raised to the ¾ power) and assumptions of linear toxicokinetics [19,22,23]. Improving high-to-low dose extrapolation is particularly important because Cr(VI) drinking water concentrations that induced tumors in rodents ($\geqslant$20 mg Cr(VI)/L) are more than 1000-times higher than typical drinking water concentrations of Cr(VI) to which humans are exposed (0.001–0.005 mg Cr(VI)/L) [12,24].

2. Materials and methods

The published literature for chromium was reviewed to: (1) identify important toxicokinetic processes needed to develop a conceptual model; (2) identify key data sets that can be used to support development of a PBPK model; and (3) identify important data gaps for chromium toxicokinetics. With respect to the latter, the NTP cancer bioassays for chromium include tissue time-course data for total chromium [1,25]; however because the small intestines and oral cavity were not identified as target tissues prior to the completion of the Cr(VI) bioassay, concentration data for these tissues were not collected. To supplement these data, two 90-day drinking water studies were conducted for Cr(VI) in female F344 rats and B6C3F1 mice (i.e., the species and strains used in the NTP bioassays) to quantify total chromium in target tissues. Because the dose–response relationships for tumors appear similar in male and female animals, and because there is no reason to anticipate sex differences in chromium toxicokinetics, tissue measurements in both sexes were not required. Details pertaining to the conduct of these studies are described elsewhere [19,20]. In brief, animals were exposed to Cr(VI) in drinking water for 90 days at concentrations similar to those in the NTP cancer bioassay (5–180 mg Cr(VI)/L) and two lower concentrations (0.1 and 1 mg Cr(VI)/L) as SDD. Target tissue concentration data in the low-dose range should provide internal dose information around the point of departure for characterizing cancer potency. Chromium cannot be speciated in tissues, and thus total chromium content was determined in the following tissues: oral mucosa, duodenum, jejunum, ileum, stomach, plasma, erythrocytes, and liver. Because animals in the NTP cancer bioassay became anemic [1], and because there are potential interactions between the toxicokinetic processes for chromium and iron, iron content was also determined in the same set of tissues. Tissue chromium and iron concentrations were first tested for dose-related trends using Jonckheere's test [26]. Datasets with a significant trend were then analyzed by Williams' (parametric) or Shirley's tests (nonparametric) [27], whereas Dunnett's (parametric) or Dunn's (non-parametric) tests were run if a monotone trend was not observed. Statistical tests were performed using the open-source, programming language, R (http://www.R-project.org). Additional data were collected to support model development for the *ex vivo* reduction of Cr(VI) in gastric contents. The methods and results for this study are described elsewhere [21,28].

Code for the PBPK model was developed to describe the key toxicokinetic processes identified in the conceptual model. All PBPK modeling was performed in 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 identified in Step 1; and (3) professional judgment. Data from the published literature in contained within figures were converted to numerical values using