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Use of a simple pharmacokinetic model to characterize exposure to perchlorate

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A simple two-compartment first-order pharmacokinetic model that predicts concentrations of perchlorate in blood and urine was constructed and validated. The model was validated using data from a high-dose experiment in humans where doses and resulting concentrations of perchlorate in blood and urine were well documented. Specifically, data were available for individuals who had been dosed at 0.5, 0.1, and 0.02 mg/kg/day for 14 consecutive days, significantly higher than the average background dose, which is estimated to be less than 0.0001 mg/kg/day. The average measured urine concentration in the high-dose regime during the experiment was 15.4 mg/l compared with an average prediction of 17.3 mg/l. In the medium-dose regime, the average measured was 3.0 mg/l compared with 4.1 mg/l predicted, and in the low-dose regime, the average measured was 0.53 mg/l compared with 0.68 mg/l predicted. For blood, the analogous results include 0.51 mg/l measured compared with 0.54 mg/l predicted in the high-dose regime and 0.12 mg/l measured *versus* 0.11 mg/l predicted in the medium-dose regime. The model was then used to study background exposures to perchlorate. A national sampling of perchlorate in urine showed a median concentration of 0.0035 mg/l, and this was used to back-calculate a dose of 0.000064 mg/kg/day. This finding was independently verified with the modeling structure of this study, as use of that back-calculated dose of 0.000064 mg/kg/day resulted in predictions of urine concentration with an average virtually identical at 0.0033 mg/l. An examination of literature data on the possible pathways of exposure suggests that the consumption of foods, rather than ingestion of water, dominates background exposures. Daily variation in urine concentration was studied with the model, and it was found that concentrations in the morning hours were lower than concentrations in the afternoon and evening hours, corresponding to the time when most exposure was assumed to occur.
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Introduction

Perchlorate (ClO_4^-) has been found in drinking water supplies (EPA, 2007), and recent surveys have also suggested widespread low-level occurrence in the food supply (FDA, 2007; Murray et al., 2008). It is a component in ammonium perchlorate (NH_4ClO_4), which is an oxidant used in missile/rocket propellants (EPA, 2007). It occurs naturally in limited circumstances and is found in Chilean fertilizer used on citrus crops (EPA, 2007). The principal health concerns associated with exposure to perchlorate are iodide uptake inhibition and reduced thyroid hormone production. The National Academies of Science (NAS) identified the fetuses of pregnant women who have hypothyroidism or iodide deficiency as the subpopulation most sensitive to the effects of perchlorate exposure (NAS, 2005). The United States Environmental Protection Agency (EPA) has established a reference dose (RfD) of 0.0007 mg/kg/day, for exposure to perchlorate (IRIS, 2007). An RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Perchlorate has been considered to be a water contaminant, but recent evidence suggests that background exposure to United States (US) citizens also occurs through consumption of food (FDA, 2007; Murray et al., 2008). The best characterization of US background exposure to perchlorate is from an analysis of results from the National Health and Nutrition Examination Survey (NHANES) conducted by Blount et al. (2007). A subset of data from NHANES, 2001–2002, includes urine samples from a nationally representative population of 2,820 US residents, ages 6 and older, which were analyzed for perchlorate content. Detectable concentrations of perchlorate were found in all samples, suggesting national exposure to perchlorate. The ion chromatography and electrospray tandem mass spectrometry analytical method (Valentin-Blasini et al., 2005) used to quantify perchlorate in the NHANES urine samples was able to achieve very low detection limits at 2.5×10^{-8} mg/l (0.025 ng/l). The median concentration found in all

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individuals in the survey was 0.0036 mg/l, with a 95th percentile concentration of 0.014 mg/l. The median and 95% concentrations of perchlorate in urine for adults only (the subset of individuals in NHANES >20 years of age, $n = 1532$) was 0.0035 and 0.012 mg/l. Using a creatinine correction approach, Blount et al. (2007) calculated doses of perchlorate of 0.000064 and 0.000234 mg/kg/day, respectively, that correspond to these urinary levels. In this approach, both creatinine and the contaminant in question are measured in spot urine samples, and based on the knowledge of the amount of creatinine excreted daily, one is able to estimate daily amounts of the contaminant to which the individual is exposed. Further information on the creatinine correction approach can be found in Cockroft and Gault (1976) and Mage et al. (2004).

The creatinine correction approach is unique in that it takes a body burden measurement and back-calculates an exposure dose that would lead to this body burden. Most efforts that attempt to link exposure dose and body burden instead take a forward calculation approach that starts with the dose and, with the use of pharmacokinetic (PK) models, predict the body burden concentration. Of course, these models can also be used in a backwards mode, predicting dose starting from body burden, but the more common use is in a forward mode to predict body burden from dose. Clewell and colleagues (Clewell et al., 2003, 2007; Merrill et al., 2005) have developed a complex, physiologically based PK (PBPK) model for perchlorate exposure in laboratory rats and humans, with an emphasis on predicting the inhibition of iodide uptake by the thyroid gland. The most recent use of this approach (Clewell et al., 2007) also included a limited verification of the model to predict human blood concentrations using data from Tellez et al. (2005).

However, this and similar PBPK models are very complicated and sometimes difficult to parameterize. For example, the perchlorate PBPK model has 14 compartments and 15 rate constants. The forward-based PK approach taken in this study is much simpler than the PBPK model for perchlorate and only focuses on predicting perchlorate concentrations in blood and urine. Iodide inhibition, transfer of perchlorate through multiple organs (gut, kidney, liver, skin, etc.), and other capabilities of the perchlorate PBPK model are not included in this approach. It is a simple, two-compartment first-order model that is easily implemented on an Excel or similar spreadsheet. The two compartments are blood and bladder. An ingested dose of perchlorate is assumed to instantaneously mix with blood and then to dissipate into the bladder assuming a first-order loss rate constant. The full amount residing in the bladder is voided with each urination. The model is an extension of the blood concentration model provided in Crump and Gibbs (2005). The capability of the model to also predict breast milk concentrations of perchlorate is examined below.

Similar one-compartment first-order PK models have been developed and applied to persistent and bioaccumulating contaminants such as dioxin (Lorber, 2002; Aylward et al., 2005) and polybrominated diphenyl ethers (Lorber, 2008). As noted above, the creatinine correction approach has been applied to perchlorate (Blount et al., 2007) and also recently to pesticides (Mage et al., 2004). The primary benefit of these simple models is that they allow for an expanded study of the magnitude and patterns of external exposure. This is a different emphasis than the complex, PBPK models, which are used more to study the internal fate (e.g., dose-to-target organs) and potential health effects of contaminants to which an individual is exposed. With the growing relevance and use of NHANES to characterize background body burdens for an expanding list of contaminants, there is a concurrent need to determine the patterns of background exposures that lead to these body burdens. For example, once quantifying the background exposure dose of perchlorate, the analysis in this paper then looks at the potential pathways of exposure that could lead to this dose and concludes that food is the primary pathway of perchlorate exposure. Exposure doses determined with simple models can be compared with health benchmarks such as the RfD, which are expressed on a dose basis. In short, these simple models are allowing for an expanded and more valid study of external patterns of exposure.

Model description

Crump and Gibbs (2005) present the following model for predicting blood concentrations from dose:

$$\begin{aligned} dC/dt &= [\text{rate into blood from gut}] - [\text{rate into urine from blood}] \\ &= [(\alpha D/V_d) * \exp(-\alpha[t - t_0])] - \beta C \end{aligned} \quad (1)$$

where C, is the concentration in blood, mg/l, t is the time, t_0 is the initial time, α is the constant reflecting active transport from amount in gut into blood, h^{-1}, D is the amount of dose per unit body weight for a given dosing event, mg/kg, V_d is the volume of distribution of blood serum per unit body weight, l/kg, β is the rate of transfer from blood to urine, h^{-1}.

Crump and Gibbs (2005) developed this model using data from the Greer et al. (2002) study. Specifically, they calibrated V_d and β by using data from one of the study participants who was dosed at a rate of 0.5 mg/kg/day of perchlorate, in four equal doses of 0.125 mg/kg/day, at 0800, 1200, 1600, and 2000 hours, for 14 days. The study in this paper uses all eight of the individuals dosed at this high rate as well as seven individuals dosed at a medium rate and four individuals dosed at a low rate. The calibrated values of V_d and β, which led to a best-fit match between predicted and observed perchlorate blood concentrations in Crump and Gibbs (2005) were 0.34 l/kg for V_d and 0.0924 h^{-1} (half-life = 7.5 h) for β. These values are used in all simulations of this paper—for the modeling of the entire Greer cohort as

well as the background simulations. It should be pointed out that the volume of distribution does not equate to the volume of serum in the human body at any one time. The volume of whole blood in the body is about 5 l and serum is about half the volume of whole blood; so the instantaneous volume of serum per unit body weight is about 0.036 l/kg (2.5 l per 70 kg). Blood flow in a resting state is about 5 l/min, hence serum flow is about 2.5 l/min, and it would therefore take about 10 min of circulation to arrive at the calibrated serum V_d for a 70 kg adult (0.34 l/kg = 23.8 l for a 70 kg adult; 23.8 l at the rate of 2.5 l/min for ~10 min). Crump and Gibbs (2005) did not provide a value for α, but for current purposes, it is neglected—it is assumed to be at a value of 1.0 meaning that any dose delivered to the gut enters into the blood instantaneously. The explanation for this assumption is that the model is implemented on a spreadsheet with a time step of 1 h. The amount of blood flow within 1 h is about six times more than the mixing volume, so an assumption of instantaneous mixing with a 1 h time step is appropriate.

Crump and Gibbs (2005) did not present results for urine concentration, although they stated that urine concentrations were simulated. They stated that there was no information on time of last void and that the results differed meaningfully based on whether the void was, as they state, "a 4-h void, a 12-h void, or an instantaneous void". As discussed below, urine data including times, volumes, and perchlorate concentrations from the Greer et al. (2002) study were available for the current effort; these data may not have been available to Crump and Gibbs (2005).

As noted, the model is implemented on a spreadsheet. A serum reservoir of perchlorate in mass units is maintained, and serum concentrations are modeled as the mass divided by V_d. Dose amounts are instantaneously added to this reservoir in the full amount. The amount of perchlorate leaving the reservoir per hour is defined as follows: (serum reservoir) $\times$ (1−exp $(-\beta t)$), where t is the model time interval—1 h. With a value of β of 0.0924 h^{-1}, the parenthetical containing the exponential term is solved as 0.088; about 8.8% of the mass in the blood reservoir exits this reservoir and deposits into a second reservoir, a bladder reservoir, each 1 h time step of the model. This second reservoir is emptied with each urination. The time and volume of urination must be specified, and the concentration in the urine is calculated as the full amount of perchlorate currently in the bladder reservoir divided by the volume of urination. Although it may be true that less than 100% of perchlorate in the bladder at any time is voided with each urination, more precise information was not available, so this simplification was made for purposes of modeling. One circumstance where it is likely that less than 100% of perchlorate is voided is when there is a residual amount of urine remaining in the bladder following urination. This happens with older individuals, but it was less likely to happen with the individuals who are modeled in this study. These individuals of the Greer study, described below, were all under 60 years of age, and only 2 of 19 individuals were above 50 years of age. Model adjustments would be necessary to consider residual urine in the bladder with incomplete urinations. It may also be true that less than 100% of ingested dose ultimately exits the body through urination; some studies have only been able to account for as little as 70% of ingested perchlorate in urine (Lawrence et al., 2000; Braverman et al., 2006).

In a lactating woman, perchlorate also deposits into her milk and exits the body. This avenue of loss will be discussed below. However, the modeling study to follow does not consider the loss through breast milk.

Overview of data and model validation procedure

The Greer et al. (2002) study includes measurements of perchlorate in both serum and urine for all of the individuals in the studied dose regimes. Other measurements, particularly relating to iodide uptake inhibition, were part of the Greer et al. (2002) study, but only the serum and urine perchlorate measurements were used in this exercise. A general description of the study is found in Greer et al. (2002), and the serum/urine perchlorate data were available in raw form from Merrill (2001). The dosing levels for three sets of study subjects in one part of the experiment, designated as high, medium, and low dosing, were 0.5, 0.1, and 0.02 mg/kg/day, respectively. The blood and urine concentration data from the high- and medium-dosing regimes were used, but only the urine data from the low dose was used because blood concentrations were mostly nondetects (NDs) for this group at a detection limit of about 1 p.p.b. It should be noted that the urine data for this low group was compromised and only a portion of it was used for this analysis. Specifically, of eight individuals in this group, data from only four were used: data were designated as "incomplete" and not supplied in Merrill (2001) for three individuals, and for the other, all observed urine concentrations appeared to be transcribed incorrectly or possibly this individual had been incorrectly dosed at a higher regime than planned. The listed measurements were all higher by just about a factor of ten compared to all other individuals in the low-dose group. For example, the first four measurements from this individual ranged from 4.6 to 9.3 mg/l, whereas the model predicted 0.6–1.0 mg/l in a limited-model application to this individual. The average concentration of perchlorate in this individual's urine overall measurements was 4.6 mg/l, whereas for all others in the low-dose group, the average was 0.54 mg/l.

Greer et al. (2002) developed data for eight individuals in the high-dose set, seven individuals in the medium-dose set, and as noted, four individuals in the low-dose group that were used in this study. The test subjects were dosed on a body weight basis. Dosing solutions were prepared by mixing pharmaceutical-grade Perchlorocap capsules, each

containing 200 mg potassium perchlorate (144 mg perchlorate), into spring water in carefully controlled amounts, stored in clear half-liter plastic bottles for the study participants to keep in their refrigerators. Participants were instructed to consume 100 ml of water, which contained the predetermined amount of perchlorate specific to their body weight and dosing regime, four times a day: 0800, 1200, 1600, and 2000 hours, for 14 days at equal amounts of 0.125 mg/kg/event for the high dose, 0.025 mg/kg/event for the medium dose, and 0.005 mg/kg/event for the low-dose groups. Urine samples were taken pretest to confirm ND, and then on exposure days (ED) 1, 2, and into day 3. After day 3, no samples were taken until ED 8 into ED 9, and then none again until ED 14. After dosing was completed, samples were continued to be taken during postexposure days (PED) 1, 2, 3, and sometimes into PED 4 if positive occurrences of perchlorate in urine were still occurring. Results for urine samples included time, volume, and perchlorate concentration. About half of the participants supplied individual urine samples and the other half supplied urination samples pertaining to 4 h periods: from 0800 to 1200 hours, for example. Although not ideal, as data from individual urination events would be preferable, these data were still appropriate for model validation purposes and for evaluating temporal variability in urine. Blood samples were taken pretest and then on ED 1, 2, 3, 4, 8, 14, and PED 1–4. Blood samples were obtained generally around 0800, 1200, and 1600 or 1700 hours on these days; some days included only one sample, whereas others included all three. Results for blood samples included exact time of sampling and perchlorate concentrations.

Blood and urine samples were analyzed at the Air Force Research Laboratory at Wright Patterson Air Force Base in Ohio. They used a method similar to that described in Narayanan et al. (2003) with the exception that they used different mobile phase concentrations: 80 mM NaOH for serum and from 60 to 120 mM NaOH for urine. Narayanan et al. (2003) describe a sensitive high-performance liquid chromatography method with lower detection limits for perchlorate in biological fluids in the range of 0.003–0.006 mg/l. Detection limits were not a problem with the high- and medium-dose data sets used in this model validation exercise, as concentrations during dosing periods were always quantified for blood and urine. As noted earlier, blood measurements were mostly ND for the low-dose group; hence the model was not validated for predicting blood concentrations for this group.

The strength of the data is clear for this model validation exercise. The doses were up to three orders of magnitude higher than typical background, as will be discussed below. Subsequently, the quantified blood and urine concentrations were substantially above background and not influenced by anything the participants would otherwise have eaten or drunk. The time of dosing was well controlled. The time and volume of urine samples were provided, and the model requires this information as input—generation of urine volumes and times of voiding are not predicted by the model. All that is required for validation of the blood concentration module is the time and concentration (not volume of blood sampled), and these data were provided.

For model validation purposes, some small adjustments and assumptions were necessary. The prediction of urine concentrations requires knowledge of the time of the previous urination event. For the resumption of urine sampling on days 8 and 14, assumptions had to be made as to the previous time when this occurred. In each case, an assumption of 3 h since previous urination if the resumption on day 8 or 14 was about 1200 hours, or up to 7 h (i.e., the time from 0000 hours the previous night) if the resumption was in the morning in the 0700 to 0800 hours time frame. For this latter resumption, it was assumed that the urination was the first void of the morning and that the individuals did not get up during the early morning hours to urinate. These assumptions are reasonable for this cohort: an examination of their urination patterns showed that they would urinate once in the 0800 to 0900 hours time frame, and there were very few early morning urinations. This latter trend is discussed further below. Urination data were provided prestudy, when dosing occurred, and for several days post dosing. The pretest showing ND was obviously not used, and only a limited number of the PED urinations were used. Specifically, PED urinations showing a positive, although of course declining, concentration were used until at least two consecutive NDs (at detection limit of about 1 p.p.b.) were measured. Often the data showed NDs within the first or second PED, and it was not useful to continue comparing predicted and measured urine concentrations once ND was established.

A small adjustment was also made in the comparison of predicted and measured blood concentrations. As noted earlier, the PK model was implemented on a spreadsheet with a 1 h time step. At the hour during which dosing occurred, the blood concentration was instantaneously elevated. Actual blood concentrations would not follow this pattern—there is a time lag between dosing and complete mixing in the blood. As blood measurements were often taken at almost very much around the same time as a dosing event, a direct comparison between "predicted" and instantaneously elevated blood concentrations is not meaningful. For example, the first blood measurement of the dosed individuals occurred near 1200 hours on day 1 of dosing. They had already taken one dose at 0800 hours and their second dose was scheduled to occur at 1200 hours. As a specific example, one individual dosed at the high amount had his/her first blood measurement at 1210 hours, and the measurement was 0.32 p.p.m. perchlorate. The model predicted concentrations of 0.37, 0.34, 0.31, and 0.28 p.p.m. for 0800 (following a dosing at 0800 hours), 0900, 1000, and 1100 hours, respectively. At 1200 hours, there was another dosing and the model

predicted a rise to 0.62 p.p.m. For purposes of comparing predicted and measured blood concentrations, the value predicted at 1100 hours, 0.28 p.p.m., was assumed to be more relevant to the observation of 0.32 p.p.m. taken at 1210 hours. The concentration at 1210 hours could have been influenced by the dose at 1200 hours (if in fact the individual dosed himself/herself at precisely 1200 hours; information on when the individual actually drank the water with perchlorate was not provided), but with a time step of 1 h, it did not appear reasonable to be comparing the predicted and instantaneously elevated blood measurements with the measurement taken near the time of dosing. This adjustment in comparing predicted and observed blood concentrations occurred at every instance where the blood measurement was taken at about the same time, but after a scheduled dosing event.

Validation results

Table 1 provides a summary of the blood and urine concentration predictions compared with measurements that were taken during the Greer experiments. Figures 1–3 compare predicted with observed individual void urine concentrations at the 0.5 (Figure 1), the 0.1 (Figure 2), and the 0.02 mg/kg/day (Figure 3) dosing regimes that were taken during the experiments. Figures 4 and 5 compare predicted with observed individual blood concentration measurements at the 0.5 (Figure 4) and 0.1 mg/kg/day (Figure 5) dosing regimes that were taken during the experiments. Figures 6–9 show how the model predicts blood concentration over time for the 0.5 (Figures 6 and 7). and 0.1 mg/kg/day (Figures 8 and 9) dosing regimes compared with measurements for four specific individuals.

These tables and figures show a good fit between predicted and measured urine concentrations. As seen in Table 1, when average urine concentrations were observed to be generally lower in the high-dose group—in the 7–13 mg/l range—predictions were also the lowest in the reasonably comparable range of 12–17 mg/l range. When measurements were higher in the 16–22 mg/l range, predictions also tracked higher in the 19–24 mg/l range, and specifically, the three highest observed average concentrations at 19.3, 21.7, and 20.8 mg/l

Table 1. Summary of urine and blood validation results by individual.

Demographics				Urine simulations						Blood simulations	
						Average concentrations (mg/l)		Cumulative mass perchlorate excreted (mg total) (14+ days)		Average concentrations (mg/l) ($n = 12$ observations)	
Identifier	Age	Wt	M/F	AV[a]	n[b]	Pred	Obs	Pred	Obs	Pred	Obs
I. *High-dosed individuals: 0.5 mg/kg/day*											
HD1	22	72	F	0.35	40	13.2	13.6	150	180	0.52	0.47
HD2	23	72.6	M	0.55	30	11.4	7.3	140	102	0.66	0.64
HD3	26	68.2	M	0.23	26	23.8	19.3	131	113	0.52	0.44
HD4	45	100.5	F	0.27	44	20.8	21.7	191	193	0.54	0.59
HD5	23	81.5	M	0.44	27	21.3	20.8	161	175	0.52	0.64
HD6	26	54.2	F	0.42	27	12.0	8.2	101	79	0.51	0.33
HD7	43	63	F	0.26	23	19.7	16.5	106	89	0.53	0.32
HD8	23	81.5	M	0.61	22	17.6	13.4	140	107	0.53	0.65
II. *Medium-dosed individuals: 0.1 mg/kg/day*											
MD1	23	70	M	0.33	25	3.7	3.9	25	29	0.11	0.15
MD2	49	75	M	0.25	38	3.7	2.5	31	22	0.10	0.09
MD3	44	67.5	F	0.29	23	5.2	1.8	27	10	0.10	0.09
MD4	26	84	M	0.43	23	4.5	4.0	30	29	0.11	0.13
MD5	25	65.9	F	0.43	25	3.4	2.7	25	23	0.11	0.16
MD6	52	106	F	0.48	28	4.2	3.1	41	33	0.11	0.15
MD7	24	86.4	M	0.36	26	4.2	3.4	29	27	0.11	0.10
III. *Low-dosed individuals: 0.02 mg/kg/day*											
LD1	34	72.7	F	0.19	41	0.77	0.52	5	3	Data not available for blood	
LD2	57	66	M	0.24	37	0.55	0.49	5	4	validation; see text for more detail	
LD3	26	106.3	M	0.59	20	0.93	0.81	8	8		
LD4	45	86.2	M	0.81	21	0.50	0.32	6	5		

[a]AV, average volume per void in liters.
[b]n, total number of urinations involved in the validation.

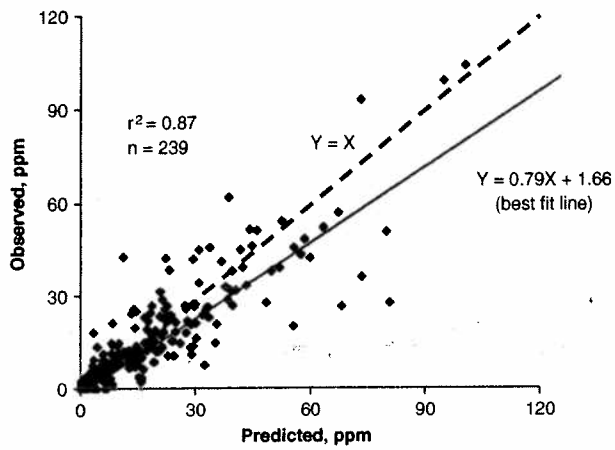

Figure 1. Predicted *versus* observed perchlorate urine concentrations for 0.5 mg/kg/day dosing (observed data from Merrill, 2001).

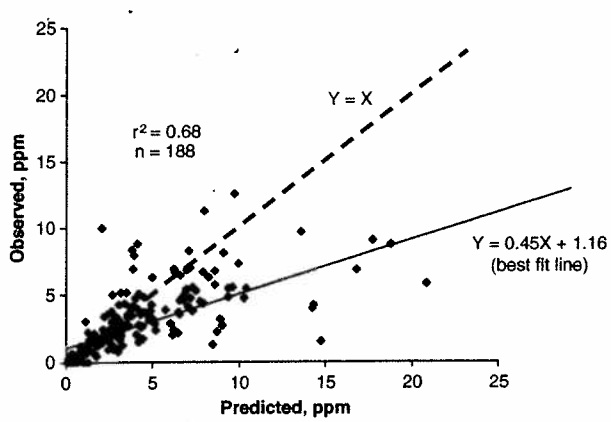

Figure 2. Predicted *versus* observed perchlorate urine concentrations for 0.1 mg/kg/day dosing (observed data from Merrill, 2001).

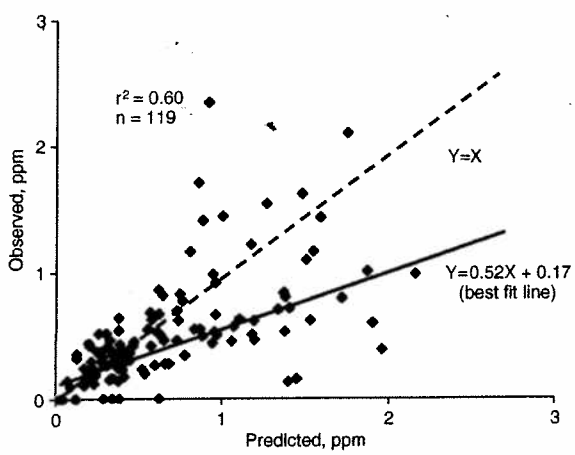

Figure 3. Predicted *versus* observed perchlorate urine concentrations for 0.02 mg/kg/day dosing (observed data from Merrill, 2001).

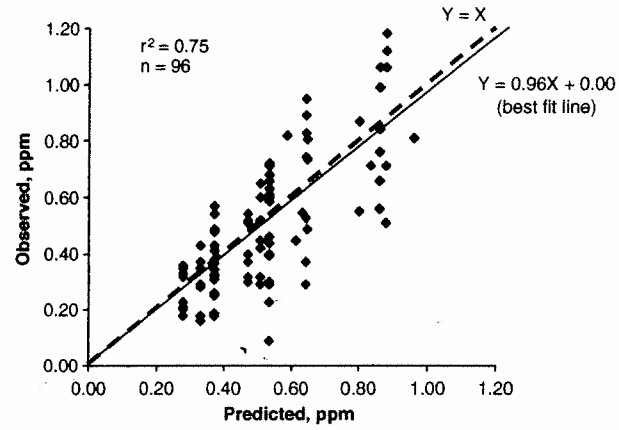

Figure 4. Predicted *versus* observed perchlorate blood concentrations for the 0.5 mg/kg/day dosed individuals (observed data from Merrill, 2001).

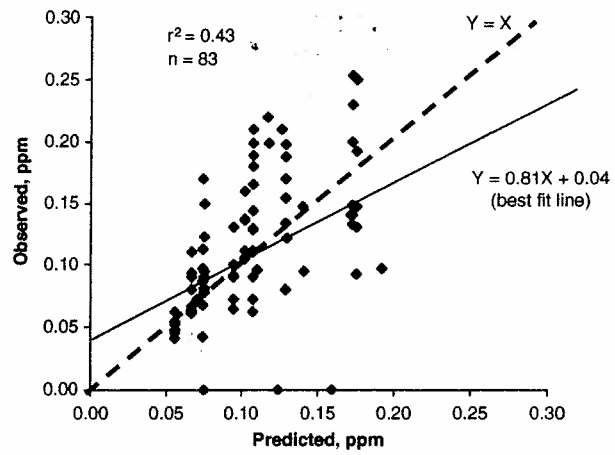

Figure 5. Predicted *versus* observed perchlorate blood concentrations for the 0.1 mg/kg/day dosed individuals (observed data from Merrill, 2001).

were matched with predictions of 23.8, 20.8, and 21.3 mg/l, respectively. Predicted urine concentrations tended to be slightly higher than measurements for this high-dosed group; Figure 1 shows that, for the best-fit line in the graph showing predicted and observed urine concentrations, observed concentrations (the Y values) were about 80% of the predicted concentrations (the X values), with an r^2 of 0.87. In all 239 observations, the predicted concentration for this high group was 17.3 mg/l compared with a measured 15.4 mg/l.

The overprediction was more pronounced for the medium-dosed group—the average prediction in all 188 events was 4.1 mg/l compared with an observed average of 3.0 mg/l. However, this result is skewed by one individual. The observed average concentration for individual "MD3" was

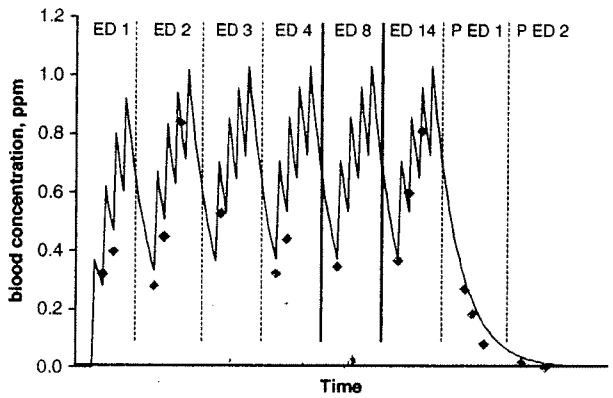

Figure 6. Predicted (solid lines) and observed (diamonds) perchlorate blood concentrations for test subject "HD1" dosed at 0.5 mg/kg/day (observed data from Merrill, 2001; ED, exposure day; PED, postexposure day).

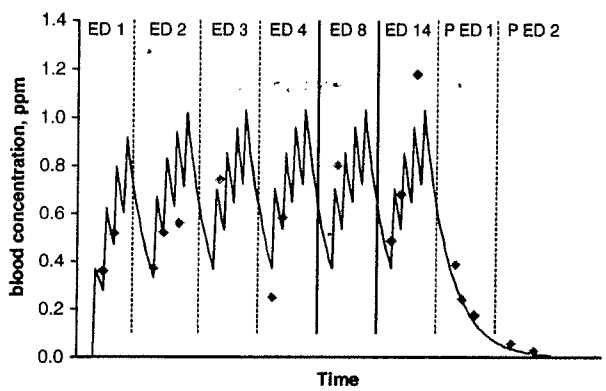

Figure 7. Predicted (solid lines) and observed (diamonds) perchlorate blood concentrations for test subject "HD4" dosed at 0.5 mg/kg/day (observed data from Merrill, 2001; ED, exposure day; PED, postexposure day).

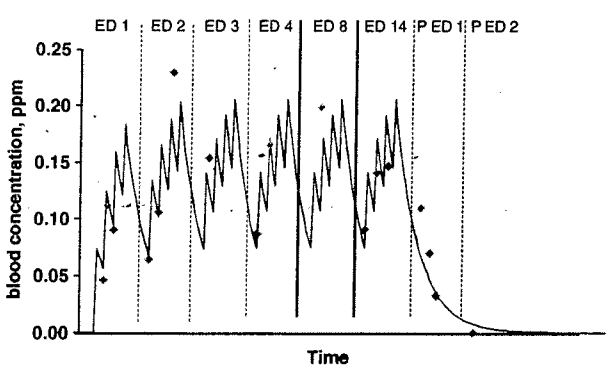

Figure 8. Predicted (solid lines) and observed (diamonds) perchlorate blood concentrations for "MD4" dosed at 0.1 mg/kg/day (observed data from Merrill, 2001; ED, exposure day; PED, postexposure day).

1.8 mg/l whereas the predicted average was 5.2 mg/l. If these observations are deleted, then the average predicted concentration drops to 3.9 mg/l, and the average observed

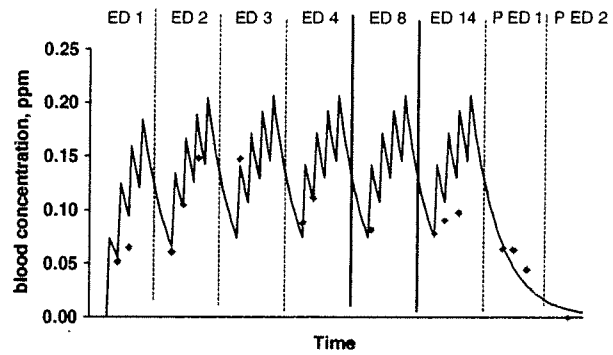

Figure 9. Predicted (solid lines) and observed (diamonds) perchlorate blood concentrations for "MD2" dosed at 0.1 mg/kg/day (observed data from Merrill, 2001; ED, exposure day; PED, postexposure day).

concentration increases to 3.2 mg/l. The r^2 also improves with this individual being removed—it was 0.68 in all observations but increased to 0.75 when this individual was removed. The best-fit line not including this individual has a slope of 0.53, but an intercept of 1.10 mg/l; when forced through the origin (i.e., at the point where the x axis crosses the y axis at (0,0)), however, the slope increases to 0.68, meaning that observed concentrations are, on average, 0.68 times predicted concentrations.

It is not clear why this individual's perchlorate urine concentration was so much lower than other individuals in this set and so much lower than predicted. An examination of the individual data from this test subject shows a consistent pattern of overprediction—there was not only a few events which were severely overpredicted. So although the match between predicted and observed urine concentrations for the medium-dosed group does not seem as robust as the match for the high-dosed group, the average predictions and measurements appeared to range narrowly in the 2–5 mg/l range.

The results for the low-dose group are consistent with the other two groups: higher-modeled urine concentrations (e.g., 0.93 mg/l average for the individual labeled "LD3") tracked with higher measured urine concentrations (0.83 mg/l for LD3), and overall, the predicted average concentration of 0.68 mg/l was similar to the measured average concentration of 0.53 mg/l.

Table 1 also shows cumulative excreted perchlorate in urine (i.e., the total amount of perchlorate excreted over the course of the 14-day experiment). The trends generally and between individuals appear well captured: the highest predicted cumulative excretion matches the highest observed excretion, and similarly for the lower excretions. For example, the highest observed cumulative amount of perchlorate excretion in the high-dose group was 193 mg, compared with a predicted cumulative excretion of 191 mg. In contrast, the two lowest observed cumulative excretions in this high-dose

group were 79 and 89 mg, compared with predicted cumulative excretions of 101 and 106 mg, respectively.

For this data set, it appears that there are no metabolic or retention differences among the three dose regimes. Indeed, there appears to be essentially a linear response in dose and urine concentration. The ratio of measured urine concentration to dose is very similar for all three dose regimes—it is about 0.03: (0.5 mg/kg/day)/(15.3 mg/l) = (0.1 mg/kg/day)/(3.0 mg/l) = (0.02 mg/kg/day)/(0.53 mg/l) = 0.03. There is no reason to believe that this would not also be true with background exposures, even though background exposures are orders of magnitude lower than the Greer dose regimes. This supports the use of the model to characterize background exposures, as is done below.

Further insight into the comparability of predicted and observed urine concentrations, and also general trends in temporal variability, can be gained by grouping the void events into specific time frames. Table 2 shows the results of this grouping, which was done for the high-dose group only. The data was grouped into six different time periods of a day: between 0100 and 0400, 0500 and 0800, 0900 and 1200, 1300 and 1600, 1700 and 2000, and 2100 and 0000 hours These results are only for the 14 days while individuals in the high-dose group were dosed; this is to avoid a skewing of the data because of lower urine concentrations while the body was ridding itself of perchlorate when dosing stopped. Although these postexposure day measurements were useful for model validation, they are less so for trend analysis. Because of the deletion of these values, there were only 155 measurement/predictions for use as shown in Table 2 instead of the 239 as in Table 1.

A clear temporal trend in the data is seen in Table 2. The doses occurred at 0800, 1200, 1600, and 2000 hours. The highest observed concentrations in the range of 21–27 mg/l occurred for results specific to the pm hours, 2100 to 0000 hours. This corresponds to the time after most of the dosing: specifically three of the four doses influence urinations that occurred within this time frame—the 1200-, 1600-, and 2000-hour doses. In contrast, the concentrations in the am hours— from 0100 to 1200 hours—are the lowest ranging from 10–20 mg/l. Only one dose occurred during this time, at 0800 hours. With such a short half-life in blood, at 7.5 h, it is logical that the body would rid itself of perchlorate within hours of exposure. Kirk et al. (2007) found this same trend with breast milk concentrations: they found lower concentrations in morning samples as compared with evening samples taken after dinner. Temporal predictions of urine concentration track reasonably well with observations. There was a consistent overprediction, but the am *versus* pm hour trend holds, and the two observed lowest concentration time frames of 0100 to 0400 and 0900 to 1200 hours were also the two predicted lowest concentration time frames.

Table 2. Temporal trends in predicted and observed urine concentrations of perchlorate for the high-dose group.

Time frame	n	Average void volume (l)	Average concentration (mg/l)	
			Pred	Obs
0100 to 0400 hours	5	1.01	14.5	10.1
0500 to 0800 hours	25	0.42	25.1	20.3
0900 to 1200 hours	34	0.35	14.5	14.9
1300 to 1600 hours	30	0.35	22.5	21.8
1700 to 2000 hours	31	0.35	29.5	24.2
2100 to 0000 hours	30	0.37	30.1	27.3

This group was dosed at 0.5 mg/kg/day at four equal doses of 0.125 mg/kg/day at 0800, 1200, 1600, and 2000 hours.

This temporal trend has implications about interpreting urine concentration data. Looking only at observed concentrations, the weighted average concentration (weighted only by number of samples taken, not by volume of urine) for samples taken in the am hours is 16.6 mg/l, whereas the weighted average concentrations for samples taken in the pm hours is 24.4 mg/l and for all day is 21.2 mg/l. Therefore, urine measurements taken in the am hours will likely underestimate daily average concentration, whereas urine measurements taken in the pm hours will likely overestimate daily average concentration. This could be an issue if a urine sampling program included results only from the afternoon or from the morning, for example. Sampling programs that involve random (in time) sampling could result in a good representation of average daily concentrations, if enough samples are taken at all times of the day.

The comparison between predicted and measured blood concentrations have to be considered tentative because of the limitations of the model, and the likely uncertainty of the data in some circumstances. With a 1 h time step in the spreadsheet implementation, judgment had to be used in matching up predicted and measured concentrations, and even after that matching, the measurements were often taken at half past an hour, although the prediction is at best what might be true for exactly the hour. Also, one must assume that the individuals correctly dosed themselves at precisely the hours they were directed to (there was no information on study participant compliance). Finally, and perhaps most importantly, it should be understood that the blood concentration predictions were identical for each individual within a dosing group in this exercise. This is because both the volume of distribution parameter, V_d, and the dose itself, D, are both body weight-based. That is, the model will predict the same concentration because both the dose and mixing volume track identically—individuals of higher body weight get proportionally higher doses and proportionally higher mixing volumes into which this dose mixes. The removal of perchlorate from the blood into the bladder is proportionally the same between individuals because of the use of a constant dissipation rate, which is a function of the mass in the blood reservoir. However, the actual mass leaving

the blood reservoir is higher for individuals of higher body weight, which explains why there can be up to a factor of two difference in modeled urine concentrations. The differences in average predicted blood concentrations shown in Table 1 are due to the fact that concentrations pertaining to different hours were chosen as the actual time of blood sampling differed among individuals.

Given these caveats, there appeared to be a reasonable match between predicted and observed blood concentrations based on data displayed in Table 1 and Figures 4 and 5. Unlike predictions of urine concentrations, there does not appear to be any kind of consistent under- or overprediction: some measurements were overpredicted (as discussed below), whereas others were underpredicted. Figure 4, showing a comparison of predicted and observed blood concentrations in the high-dosed group, shows as many over- as underpredictions, with a best-fit slope of 0.96 and the best-fit intercept in fact going through the origin. The overall r^2 of 0.75 shows a reasonable fit. In all 96 observations, the average prediction was 0.54 mg/l and the average observed was 0.51 mg/l.

It is noted that there appeared to be a meaningful overprediction of two individuals, "HD5" and "HD6", as seen by blood concentration predictions about 0.52 mg/l, whereas average observations were about 0.33 mg/l. What is interesting is that there was also a noticeable overprediction in urine concentrations for these individuals: 12.0 predicted *versus* 8.2 mg/l observed and 19.7 predicted *versus* 16.5 mg/l observed. Perhaps both of these individuals were underdosed. There was an additional individual with this same trend: blood concentrations were overpredicted at 0.52 mg/l *versus* 0.44 mg/l, and urine concentrations were overpredicted at 23.8 *versus* 19.3 mg/l. On the other hand, one individual's urine concentration was overpredicted, 17.6 *versus* 13.4 mg/l, whereas this person's blood concentration was underpredicted, 0.53 *versus* 0.65 mg/l. So whereas there are individual results that appear to support a trend, there are others that do not follow this trend; therefore, perhaps it is best to derive conclusions based on this cohort as a whole.

The medium-dosed group had predicted and observed average blood concentrations between 0.09 and 0.16 mg/l. There appears to be a slight tendency towards underprediction, with four individuals having predicted average blood concentrations 0.02–0.05 mg/l lower than observed. In all sampling points, the average predicted concentration was 0.11 mg/l and the average observed was 0.12 mg/l. Figure 5 displays a lower r^2 at 0.43 and a best-fit line showing a slope of 0.81 and an intercept of 0.04. However, when forcing the line through the origin, the best-fit line has a slope of 1.1, meaning that observations would be 1.1 times predictions.

The suitability of the model to predict blood concentrations might be better characterized by showing a continuous simulated blood concentration compared with spot measurements, as displayed in Figures 6–9. These show results for four specific individuals: two in the high-dose group and two in the medium-dose group. The blood concentrations are predicted to spike up with each dose and depurate between the last dose at 2000 and the next one at 0800 hours the following morning. These figures show the difficulty of this type of matching—the exact time of either dosing or sampling of blood was not known with certainty or matching was rigorously modeled with a 1 h time step. There were occurrences of measured concentrations higher than anything modeled as well as lower than anything modeled. The trend showing that the concentration sampled most recently in the pm hours of the day was the highest appears both in the modeled and measured data. The overall range of concentrations seemed adequately captured. The pattern of depuration after the 14th day of dosing appeared to be very well modeled in all four individuals.

Use of the model to characterize background exposures

Once validated, practical applications of predictive models involve simulations of real world situations of particular interest. A better understanding of background exposures to perchlorate is sought using this model. The best characterization of background exposure to perchlorate is from an analysis of results from NHANES conducted by Blount et al. (2007). As discussed in the Introduction, a creatinine correction approach was used to characterize median adult exposure doses in this population, resulting in a predicted dose of 0.000064 mg/kg/day. It is noted that Blount et al. (2007) expresses dose and concentrations using mass units of micrograms (μg) instead of milligrams (mg) as has been done in this study because of the much higher dose and resulting blood and urine concentrations of the Greer experiments. To be consistent with Blount et al. (2007), and also to avoid large numbers of zeros in the displayed numbers, mass units of μg will be used in this discussion of background exposures.

This backward-calculated dose of 0.064 μg/kg/day can be verified with a forward calculation using the framework in this paper. Rather than construct a background population from scratch, perhaps individuals in the Greer study group can be used, as times and volumes of urination are already provided. This would be the only characteristic of the Greer study subjects used—the times and volumes of urination, not the doses of perchlorate. For this analysis of background exposures, a unique "background" cohort was constructed from the high-dose group of the Greer study. Only days in which a full day's worth of urination were included (a few of the dosing days included only one or two urinations like days 8 or 9), and also some of the PED were added to each individual because they included a full day's worth of urination. The resulting background cohort included 8 individuals and 57 complete days of urinations. The patterns

of urination in this constructed background cohort were as follows:

- 4.7 urinations/day (range of 2–7)
- an average individual void volume of 0.37 l
- an average daily total voiding of 1.73 l.

This group does not represent the ideal background cohort for at least two important reasons: (1) there were only eight individuals and obviously this cohort does not span the range of individuals with regard to age, sex, ethnicity, or other important facets that control volumes and frequencies of urination for a general population and (2) even for these individuals, their urination pattern may have been influenced by the ingestion of 100 ml of water at the four dosing times during the day. Also, as noted earlier, some of the voids were 4 h samples taken by the study participants and were not necessarily individual events, but they should still reasonably represent temporal trends and daily totals. Still, having these times and volumes of urination provided an opportunity to employ the model for situations other than the high-dose regime of the Greer experiments. A final but important change was that the dosing regime was changed to five (instead of four) equal doses of 0.02 μg/kg/day at 0900, 1200, 1500, 1800, and 2100 hours for a total of 0.1 μg/kg/day. This spreads out the dose more during the day, and also urinations at 0800 hours can be considered "first morning voids" for purposes of further evaluation.

Temporal results from this background cohort are shown in Table 3. Interestingly, the average urine concentration resulting from the daily dose of 0.1 μg/kg/day is 5.2 μg/l, and if extrapolated to a daily dose of 0.064 μg/kg/day, the concentration is 3.3 μg/l, which is essentially identical to the 3.5 μg/l from the NHANES data. Also seen in Table 3 is that the temporal trends identified from the high-dose group shown in Table 2 and discussed earlier are repeated here: the concentrations during the am hours are lower than the pm hours. This is not surprising, as the individuals modeled in Tables 2 and 3 and their patterns of urination were essentially the same; the only differences are that the pattern of exposure in Table 3 is a little more disperse as compared with Table 2 and that Table 3 includes some additional days of evaluation for the modeled individuals.

Table 3. Temporal trends in urine concentrations from the constructed background "cohort".

Time frame	*n*	Average void volume (l)	Average predicted concentration (μg/l)
0100 to 0400 hours	4	0.48	4.3
0500 to 0800 hours	48	0.43	6.1
0900 to 1200 hours	55	0.32	3.2
1300 to 1600 hours	59	0.35	4.7
1700 to 2000 hours	53	0.31	6.4
2100 to 0000 hours	53	0.45	6.0
Average		0.37	5.2

This group was dosed at 0.1 μg/kg/day, at five equal doses of 0.02 μg/kg/day at 0900, 1200, 1500, 1800, and 2100 hours (note that background exposures are expressed in lower mass units of μg/kg/day, as compared with the experimental dose range of mg/kg/day).

It is noted, from both tables, that the voids during the 0500 to 0800 hours are comparable with the afternoon void concentrations. These represent first morning voids, and as such, one might expect them to be lower, as the time of last exposure was the previous evening at 2000 to 2100 hours. For the background cohort in Table 3, they were intentionally made into morning voids by not allowing a dose until 0900 hours instead of 0800 hours as they were for the experimental conditions in Table 2. The reason that the first morning void concentrations were similar to afternoon voids near the time of exposure is clear, however: the concentration is a function of not only higher blood concentrations depositing more perchlorate in the "bladder" reservoir as in the pm hours, but also of the volume of urine and frequency of urination that rid the bladder of its current reservoir. It can easily be seen in Tables 2 and 3 that this cohort did not experience much early morning voiding, only 4–5 events between the 0100 and 0400 hours over the entire cohort. Therefore, while the first morning voids generally had higher volumes/event than the later morning or afternoon voids, they included the perchlorate that had deposited into the bladder since before midnight without being voided. The same general trend of very few early morning voids was also seen in the medium-dosed group: there were only six recorded voids between 0100 and 0400 hours for the seven tested individuals. It should be noted that the study subjects were not monitored during the study for compliance—there may have been more early morning voids than recorded. Also, voids may have been incorrectly recorded. However, the finding of higher concentrations in both the measurements in the test subjects and the modeled background cohort suggests that infrequent early morning voids was the cause of higher measured and modeled first morning voids. Perhaps, in a larger background cohort including an appropriate subpopulation of middle-aged individuals who get up frequently to urinate during the evening and early morning hours, first morning void concentrations would be lower.

One can look at the pattern of early morning voids (0100 to 0400 hours) in the constructed background cohort more closely for further insight. For these 4 days, the individuals who had early morning voids had a total of five voids between 0500 and 0800 hours (one individual had two voids; the other three had one void each). The average perchlorate concentration of these five voids was 3.8 μg/l, lower than the overall average per urination of 5.2 μg/l as well as the pm hour averages in the 4.7–6.4 μg/l range. In other words, for

those individuals with at least one void during the early morning hours, the disparity between morning and afternoon voids is more clear, and also appropriate given the toxicokinetics of perchlorate.

In summary, the constructed background cohort as well as the study cohorts linearly extrapolated downward have provided an independent verification of the Blount et al. (2007) finding that a urine concentration of about 3.5 μg/l corresponds to an exposure dose of about 0.064 μg/kg/day. Also, a temporal evaluation of the modeling results show that concentrations tend to be lower in the hours between 0100 and 1200 hours as compared with 1300–0000 hours.

The question arises as to how this exposure occurs. Based on perchlorate properties and sources, certainly general background exposures are not occurring from air or soil/dust; typical background exposure is oral exposure, by food and water (EPA, 2007). However, there remains the question of how much exposure can be attributed to both of these matrices. The Food and Drug Administration (FDA) have recently completed two food surveys, which show widespread low-level occurrence in the food supply (FDA, 2007; Murray et al., 2008).

In one of those two efforts, FDA conducted exploratory surveys from October 2003 to September 2005 to determine the occurrence of perchlorate in a variety of foods (FDA, 2007). The 27 food products tested were mostly fruits and vegetables as well as bottled water and milk, and although sampling was widespread, there was a focus on areas of the country where water impacts from perchlorate were expected. In its final release of these data in 2007, FDA also included associated estimates of exposure. Briefly, their extrapolation to all individuals greater than 2 years old resulted in an average perchlorate dose from these food products of 0.053 μg/kg/day (FDA, 2007). This survey was supplanted by FDA's recently completed Total Diet Survey (TDS), and the measurement of perchlorate in the total diet (Murray et al., 2008). The TDS consists of sampling and evaluating approximately 280 different foods and beverages, including about 60 baby foods, using a "market basket" approach. Using these data and consumption rates of food from USDA's Continuing Survey of Food Intake by Individuals (CSFII), FDA developed estimates of average dietary perchlorate intake for the total US population, including infants aged 6–11 months, children aged 2 and 6, and for several age ranges of children and adults. For all age categories of teenagers (starting at age 14) through adults, the range of exposures were 0.05–0.13 μg/kg/day. The back-calculated dose of 0.064 μg/kg/day by Blount et al. (2007) using NHANES data for adults falls within the range of exposures determined from these FDA food surveys.

Meanwhile, other evidence suggests that water is very likely to contribute a small part of this average background dose. EPA reported on the results of a nationwide survey of public drinking water systems sampled between 2001 and 2005 as part of its 1999 Unregulated Contaminant Monitoring Regulation (EPA, 2007). With a reporting limit of 4.0 μg/l, it found that 96% of 3,865 public water systems (PWS) did not have detections at this limit. Further, only 1.9% of all samples taken (637 of 34,331 samples taken from the 3,965 systems) had positive detections of perchlorate. The average concentration of perchlorate for those positive samples was 9.85 μg/l and the median concentration was 6.40 μg/l. The average tapwater intake rate recommended by EPA's *Exposure Factors Handbook* (EPA, 1997) is 1.4 l/day. Therefore, average adult exposure at the reporting limit of 4.0 μg/l is 0.08 μg/kg/day ((1.4 l/day × 4 μg/l)/70 kg), so 98.1% of all drinking water exposures (using statistic taken from the 1.9% of samples instead of the 4% of systems with at least one detection statistic) are less than this amount. The state of California conducted drinking water surveys with a similar detection limit and similar results. In 1999, the California Department of Health Services (CA DHS) began monitoring for perchlorate in drinking water sources that were identified as vulnerable to perchlorate contamination (CA DHS, 2006). About 60% (or 7,100) of all drinking water sources in California (about 12,000) were monitored between 2001 and 2006, and 284 (about 4%) sources had at least 2 or more positive detections for perchlorate at concentrations greater than or equal to the reporting limit of 4.0 μg/l. It would be preferable to have lower reporting limits to be more informative, as the total background exposure is about 0.1 μg/kg/day, near the reporting limit exposure of 0.08 μg/kg/day.

However, there was at least one reported survey that did have a lower detection limit at 1.0 μg/l. In 2005, the State of Massachusetts's Department of Environment Protection (MA DEP, 2006) used a modified version of EPA Method 314.0 and achieved this detection limit. They reported monitoring results for 85% (379 of 450) of its community water systems and 86% (212 of 250) of its nontransient, noncommunity water systems. They found that 9 (1.5%) of the 591 PWS had at least one detection of perchlorate at levels greater than or equal to 1.0 μg/l. At least for the individuals drinking water from the large majority of these systems, it would seem that their exposures through drinking water are, on average, less than 0.02 μg/kg/day.

Breast milk impacts

Several studies have measured perchlorate in human (Kirk et al., 2005, 2007; Tellez et al., 2005; Pearce et al., 2007) as well as cow milk (Kirk et al., 2005; FDA, 2007; Murray et al., 2008). Clearly, ingested perchlorate can be excreted in both breast milk and urine. In the construct of the simple model in this study, it would seem appropriate simply to apportion an amount of perchlorate that leaves the blood reservoir to deposit into each of the two reservoirs, bladder

and breast milk. Similar to the urine concentration prediction, one would input times and volumes of breast milk excretion to predict breast milk concentrations. There would be only one additional parameter in this approach: a fraction that splits the amount leaving the blood reservoir to go into two different reservoirs. However, there may be a need to reassign values of the two existing model parameters, V_d (volume of distribution in blood) and β (rate of dissipation from blood). Clewell et al. (2007), for example, calibrated a key PBPK model parameter, a urinary clearance value, when applying their model to pregnant and nursing women in Chile.

Putting aside this question as to whether V_d and β need to be reassigned in a lactating woman, there is still the question of how to assign a value that apportions dissipating perchlorate to either the bladder or breast milk reservoirs. If it can be assumed that the apportionment to urine and breast milk correlates directly with the volume of urine or breast milk excreted, then one can assign a value based on the differences in volume, and ultimately, the concentration in urine and breast milk would be predicted to be the same. However, there is one reasonably robust data set that contradicts this assumption. In a sampling of both breast milk ($n = 49$) and urine ($n = 56$, 49 of which also had milk measurements) from lactating women from Boston, Pearce et al. (2007) found that breast milk concentrations were about a factor of three higher than urine concentrations: median of 9.1 μg/l and mean of 33 μg/l in breast milk, and median of 3.0 μg/l and mean of 8.2 μg/l in urine. Interestingly, as a possible generalization, the volume of milk excretion might be about one-third as much as urine. The volume of urine excreted daily from the background cohort developed above was 1.73 l. According to EPA's *Child-Specific Exposure Factors Handbook* (EPA, 2006), a 12-month average of daily breast milk consumption by infants is 0.64 l/day. If perchlorate concentrations are about three times higher in breast milk compared with urine and if breast milk excretion is about one-third that of urine, then this would imply that about half of the ingested perchlorate goes each to breast milk and urine. That simple assumption of 50% is actually consistent with a rat PBPK modeling finding by Clewell et al. (2003), who found that at low levels of maternal exposure to perchlorate, a nursing rat pup would transfer up to 50% of the maternal intake to the infant.

In summary, it appears that the addition of a second reservoir, which holds perchlorate that would be excreted with breast milk, might work in this model construct. Limited data suggest that ingested perchlorate splits almost evenly on a mass basis between breast milk and urine, and given that urine volume tends to be up to three times higher than breast milk excretion, predicted breast milk concentrations would be about three times higher than urine concentrations. Without data to validate a breast milk portion to this simple model, however, this is as far as the analysis can go.

Summary of findings

The primary findings from this work are as follows:

(1) A simple two-compartment first-order PK model that predicts concentrations of perchlorate in blood and urine was constructed and validated using data from Greer et al. (2002). The good match between predicted and measured concentrations allows for use of the model in circumstances other than those duplicated by the validation data. The model requires as input perchlorate dose timing and amounts in mass per body weight per event as well as times and volumes of urination. The model predicts blood and urine concentrations. The model is implemented on a spreadsheet with a time step of 1 h.

(2) The model was then used to study background exposures to perchlorate. A different evaluation of background exposures based on NHANES concentrations of perchlorate in urine of 3.5 μg/l led to a finding that a median exposure dose of Americans to perchlorate was in the range of 0.064 μg/kg/day. The finding from this backward approach (estimating exposure dose from urine concentration) was independently verified in this model, as use of the same dose in a cohort constructed to simulate background exposures led to virtually the same predicted average urine concentration, 3.3 μg/l, in a forward modeling approach.

(3) Data recently released on perchlorate in food suggest that this daily background average dose can arise from food. A recently completed survey by FDA that measured perchlorate in food as part of their ongoing Total Diet Study found widespread quantified occurrences of perchlorate (Murray et al., 2008). They included estimates of dose developed with use of food consumption data and found average intakes by adults to be in the range of 0.05–0.13 μg/kg/day. The background dose of 0.064 μg/kg/day determined independently fits within this range. National and regional studies of perchlorate in drinking water find NDs in greater than 95% of samples, but use of these studies for current purposes are somewhat hampered by reporting limits of 4.0 μg/l, which correlates to an average exposure of 0.08 μg/kg/day. However, one study found greater than 98% NDs with a lower detection limit of 1.0 μg/l. With this food and water data considered together, the hypothesis is that water exposures, on average, are likely to be small in comparison with food exposures.

(4) The variation in urine concentrations over the course of a day was evaluated using the modeled and measured concentrations from the Greer et al. (2002) study as well as the background cohort. It was found that concentrations were higher nearer the time when most of the exposure was occurring and for some hours afterward. As the modeled exposures occurred mostly from 1200 to

2100 hours, the afternoon and evening concentrations were higher than the morning concentrations of perchlorate. The first morning void concentrations were themselves not necessarily higher than the afternoon and evening voids. This is because the studied cohorts mostly did not urinate during the early morning hours—from 0100 to 0400 hours. Such voids are necessary to excrete the perchlorate accumulating from afternoon and evening exposures. When they did, the concentrations were lower in their first morning voids as compared with afternoon concentrations. Because of this trend, monitoring studies focusing on only one time of day could either over- or underpredict average daily concentrations, depending on when that time was.

(5) Structurally, the model can easily be amended to include a breast milk compartment to predict breast milk concentrations. Additional model requirements would include timing and volume of milk in nursing events, and a fraction that partitions perchlorate leaving the blood to go into either the bladder or breast-milk-holding reservoirs. The data suggest that a simple assumption that the fraction is proportional to the volumes of urine *versus* breast milk is not appropriate. This is because such an assumption results in an identical concentration, but a reasonably robust study showed that breast milk concentrations were significantly higher than concurrently measured urine concentrations. Based on findings in this study in combination with the fact that daily breast milk volumes are much lower than urine volumes, it appears that perhaps perchlorate could be partitioned on a 50:50 basis—half (on a mass basis) going to each reservoir. However, without further data to validate this assumption, this is as far as the breast milk modeling could go.

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Disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the US Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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