Reconstructing exposures from biomarkers using exposure-pharmacokinetic modeling – A case study with carbaryl

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Highlights

- Computational models were used to evaluate methods for exposure reconstruction.
- Critical data needs were identified in interpreting urinary biomarkers.
- Recommendations were provided for future biomonitoring studies.

Keywords: Exposure reconstruction, Biomarker interpretation, Pharmacokinetic modeling, Physiologically Based Pharmacokinetic Model, Carbaryl, Markov Chain Monte Carlo, Discretized Bayesian, Exposure Conversion Factor, CARES, Population-based Biomonitoring.

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ABSTRACT

Sources of uncertainty involved in exposure reconstruction for short half-life chemicals were characterized using computational models that link external exposures to biomarkers. Using carbaryl as an example, an exposure model, the Cumulative and Aggregate Risk Evaluation System (CARES), was used to generate time-concentration profiles for 500 virtual individuals exposed to carbaryl. These exposure profiles were used as inputs into a physiologically based pharmacokinetic (PBPK) model to predict urinary biomarker concentrations. These matching dietary intake levels and biomarker concentrations were used to (1) compare three reverse dosimetry approaches based on their ability to predict the central tendency of the intake dose distribution; and (2) identify parameters necessary for a more accurate exposure reconstruction. This study illustrates the trade-offs between using non-iterative reverse dosimetry methods that are fast, less precise and iterative methods that are slow, more precise. This study also intimates the necessity of including urine flow rate and elapsed time between last dose and urine sampling as part of the biomarker sampling collection for better interpretation of urinary biomarker data of short biological half-life chemicals. Resolution of these critical data gaps can allow exposure reconstruction methods to better predict population-level intake doses from large biomonitoring studies.

1.1 INTRODUCTION

Biomonitoring is a relatively efficient and cost-effective means in which to measure compounds or their metabolites in blood, urine, or other specimen samples (CDC, 2009a; NRC, 2012). Biomonitoring is often used to track changes in exposures over time or to establish reference ranges for different population cohorts (e.g., gender, lifestage). Biomarkers measured in biomonitoring studies may also support risk assessment when integrated with complementary data on epidemiology, toxicity, exposure, and pharmacokinetics (NRC, 2006). One of the approaches for using biomarkers in risk assessment is to convert measured concentrations into intake doses (i.e., reverse dosimetry) for comparison against exposure guidance values already demonstrating risk connotation, such as the Environmental Protection Agency's (EPA) Reference Dose (RfD) (NRC, 2006).

Reverse dosimetry, however, is not a straightforward process. Cross-sectional biomonitoring studies such as the CDC's National Health and Nutrition Examination Survey (NHANES) (CDC, 2009a) involve taking a single spot measurement for each individual. Spot measurements reflect many interacting variables, such as timing of sample collection, as well as exposure sources, routes, magnitude, duration, and frequency. Spot measurements also reflect the variability inherent in human pharmacokinetics, namely absorption, distribution, metabolism, and excretion (ADME) of a chemical in the body. Collection of such information regarding these interacting variables, and its integration using physiologically based pharmacokinetic (PBPK) models, can aid in obtaining reasonable estimates for exposures based on biomarker data.

PBPK models can predict the time course of a chemical's and its metabolites' (if applicable) concentrations in biological tissues under various exposure and pharmacokinetic scenarios. Several research groups have demonstrated the utility of PBPK models in conducting reverse dosimetry (Allen et al., 2007; Ellison et al., 2012; Liao et al., 2007; McNally et al., 2012; Tan et al.,

2006a; Tan et al., 2006b; Ulaszewska et al., 2012). Reverse dosimetry has also been conducted using simpler pharmacokinetic (PK) models (Lorber, 2009; Lu and Andres, 2012), ratio calculations (Bartels et al., 2012) methods (Georgopoulos, 1994; Roy and Georgopoulos, 1998), or Bayesian approaches (Allen et al., 2007; Sohn et al., 2004).

Despite the large body of literature associated with using reverse dosimetry to estimate exposure concentration from biomarker data, efforts for evaluating such predictions have been hampered by the lack of corresponding measurements of biomarker data with “true” exposure conditions (Clewell et al., 2008). Exposure reconstruction is challenged by the need for inferring exposures from extremely limited information commonly gathered in large-scale biomonitoring studies (e.g., biomarker data, body weight, and urine volume) for individuals. The objective evaluation of the appropriateness of different reverse dosimetry methods, influencing determinants of dose-biomarker relationship, and errors in reconstructed dose estimates is difficult in the absence of matched exposure/biomarker measurements. As with prior exposure-dose modeling approaches (Knaak James et al., 2012), the current study utilized a combined exposure-PBPK model for carbaryl to generate corresponding time profiles of dietary intake doses and urinary biomarker concentrations in a virtual population. Exposure-dose modeling approach has been previously applied to investigate health impacts from dermal dietary exposures to an organophosphate pesticide in members of general population (Ellison et al., 2012; Hinderliter et al., 2011; Price et al., 2011). In this current study, exposure-dose modeling is used to examine sources of variability in biomarkers of exposure and identify critical data gaps that might render the ability to reconstruct intake doses from biomarker data difficult. Our proposed approach can be applied to models for a wide variety of chemicals, and here carbaryl was selected as a case study to demonstrate the approach.

Carbaryl is a widely used carbamate insecticide with a relatively short biological half-life of 9 hours (Feldmann and Maibach, 1974), whose routes of exposure include oral ingestion (via food and

water), as well as inhalation and dermal contact during application (Howard, 1991). The major metabolite 1-naphthol (1-N) is found in the urine of exposed individuals and is commonly used as a biomarker for carbaryl exposure (CDC, 2009b; Meeker et al., 2007). PBPK models for carbaryl in rats and humans have previously been developed (Nong et al., 2008; Yoon et al., 2015; Yoon et al., 2012) to predict the disposition of both carbaryl and 1-N. In addition, within-day exposure profiles (magnitude, frequency, and duration) for food and water exposure from the use of carbaryl is available from the Cumulative and Aggregate Risk Evaluation System (CARES) (ILSI, 2009) making carbaryl an ideal candidate for a case study to compare reverse dosimetry approaches and to investigate critical data needs. The two objectives of this study were to: (1) compare three PBPK model-based reverse dosimetry approaches based on their ability to predict the central tendency of the intake dose distribution; and (2) identify information necessary for a more accurate dose intake estimate from biomarker data of short biological half-life chemicals.

METHODS

Estimating dietary exposures to carbaryl using CARES

A dietary exposure model, the Cumulative and Aggregate Risk Evaluation System (CARES) Version 3.0 (ILSI, 2009), was used to estimate carbaryl exposure from food and water consumption. The CARES model has been formally reviewed and approved by the EPA's Science Advisory Panel (USEPA, 2004) and has been used by the EPA's Office of Pesticide Programs (USEPA, 2006a; USEPA, 2006b; USEPA, 2007) to estimate carbaryl intake in the general population. The CARES model combines data on food and water consumption with data on pesticide residues, such as carbaryl, in order to characterize variation in total dietary exposure in the U.S. population. CARES produces sequential estimates for periods of up to one year with a resolution of 10 minutes. CARES uses the Gower's Similarity Coefficient to identify demographic and anthropometric records that correspond to individuals with statistically similar characteristics, such as gender and age. Using this technique, year-long (365 days) dietary profiles (time-dose relationships of carbaryl exposures) were constructed for a set of simulated individuals (n=500) (Crop-Life-America, 2002).

Dietary exposure from food and water was determined based on consumption data from the Continuing Survey of food Intake by Individuals (CSFII) from 1994-1996, and 1998 (USDA, 2000). The nationwide survey indicates the time of day a food and/or meal was consumed which allows the exposure to be characterized by each meal or eating event. To allow the CSFII food consumption data to be expressed as raw agricultural commodities (RACs) or processed commodities, the Food Commodity Intake Database (FCID) was used to provide translation recipes (USEPA and USDA, 2000). Additionally, the CSFII database contains water consumption data for indirect water (i.e., water added to foods and beverages during final preparation), and for water consumed directly. A nationally representative water consumption survey has been

conducted to address how often, when, and how much water is consumed at specific times during the day (Barraj et al., 2009). These data were incorporated into CARES to give the time of day information for water consumption.

Simulating spot urinary 1-N concentrations using a PBPK model

A human PBPK model for carbaryl (Yoon et al., 2012) was used to predict the disposition of the parent chemical (i.e., carbaryl, the active species for acetyl cholinesterase [AChE] inhibition) and the principal metabolite and primary biomarker used to indicate carbaryl exposure, 1-N. The model was parameterized using human-specific *in vitro*-derived metabolic constants of carbaryl in combination with knowledge gained from modeling carbaryl kinetics and responses in the rat (see parameters used for the PBPK model in Supplementary Table 1). The PBPK model predicts the urinary concentration of total 1-N (free, plus conjugates) as reported in biomonitoring studies. For each of the 500 CARES individuals, the synthetic daily intake doses were added directly into the gut compartment of the PBPK model.

Sensitivity Analysis of the PBPK model

A local sensitivity analysis was conducted to identify PBPK parameters with the greatest influence on predicted 1-N urinary concentrations. Seven days were sufficient for the model-predicted urinary 1-N excretion to reach pseudo steady state. Three dose levels were tested: the 5th percentile, the 50th percentile, and the 95th percentile of the distribution of the largest single dose per day for all individuals (N = 365 days × 500 individuals =182,500). These doses were 0.7359, 35.09, and 154.9 ng carbaryl/kg body weight/day, respectively. The elapsed time between the final dose at each level and the time of urine sampling was also fixed at one of three values: 1, 4, or 12 h. In summary, a sensitivity analysis of all model parameters was performed for nine separate cases, with each case being a unique combination of dose level and the elapsed time between dosing and urine sampling. Model parameters (other than the one undergoing sensitivity analysis)

were set to their mean values (either the arithmetic mean or geometric mean, depending on the shape of the distribution for that variable). Normalized sensitivity coefficients were computed by dividing the change in the urinary 1-N concentration by the change in the parameter value after perturbing the value by 0.1% of its mean. Sensitive parameters were considered to be those with normalized sensitivity coefficients ≥ 0.1.

Generating the Synthetic Data for Paired Intakes and Biomarkers

Since reconstructing intermittent doses at random times from a single spot urine biomarker measurement proves difficult, the food and water exposure profiles simulated in the CARES model required simplification using two assumptions to generate synthetic daily intake doses:

(1) Each of the 500 individuals received one dose per day, for 5 days. This daily dose was the mean of 365 daily intake doses (sum of all intermittent doses within a 24 h period) from the CARES simulations, which will henceforth be referred to as the synthetic daily intake doses.

(2) Each individual received a single daily dose at the same time each day (2:42 pm) in order to consistently simulate daily intake of contaminated food or water. The time of exposure was the median of 365 time points at which the maximum dose occurred, unique to each individual.

Each of the 500 CARES individuals was assigned a unique vector of parameter values: body weight (kg) was taken from the CARES model (range from 35.6 kg to 158.8 kg, with an average of 74 kg), and sensitive parameters (results in Supplementary Table 2) were randomly chosen from their distributions (see Supplementary Table 1). These distributions were truncated at ±1.96 X σ, where σ is the standard deviation. This truncation limited sampling to approximately the central 95% of the total distribution and prevented extreme values from being sampled. All non-sensitive

model parameters were fixed to their mean (see Supplementary Table 1). This vector of sensitive and non-sensitive parameters is henceforth referred to as individual values for the synthetic individuals (known parameter values). The model was then used to predict the rate of production of 1-N in urine, *r(t)* (ng/h) as a function of time for each individual.

The model output, as a rate, required conversion to a spot urinary concentration (e.g., in ng/L), the units typically reported in biomonitoring studies. This conversion was accomplished through the use of two equations. The first equation used urine volume and the time between voids:

$$\text{Equation 1: } c(t_s) = \frac{1}{V_u}\int_{t_{s-1}}^{t_s} r(t)dt = \frac{1}{V_u}\,[m(t_s) - m(t_{s-1})],$$

where $c(t)$ is the concentration of 1-N in urine (ng/L) at time t, V_u is the volume of the urine void (L), t_s is the time of sampling (h), t_{s-1} is the time of the most recent urine void before the sampling time (h), *r(t)* is the mass flow rate of 1-N into the urine (ng/h), and *m(t)* is the cumulative amount (ng) of 1-N in urine.

An alternative equation based on urine flow rate calculated the quantity of urine produced in a specified period of time.

$$\text{Equation 2: } c(t_s) = \left({r(t_s)}/{fr}\right),$$

where *fr* is the urine flow rate (L/h).

For this study, values for urine volumes, time between voids, and urine flow rates were obtained from the NHANES 2009-2010 dataset (CDC, 2011). The two methods for calculating the urine concentration from the model output were compared (See Table 1 for simulation description summary). It was found that the predicted spot urinary 1-N concentrations using both equations were nearly identical (see Supplementary Figure 1). Thus, the second equation, which required only one additional parameter (urine flow rate) rather than two parameters (urine volume and time

between voids), was used to compute spot urinary 1-N concentrations for the synthetic individuals. For each of the 500 CARES individuals, urine flow rate was randomly sampled from the NHANES 2009-1010 dataset.

The elapsed time between the final dose and spot urine sampling was constrained to be no more than 24 h. The NHANES dataset includes a “sampling session” variable, which was used to assist in setting the time of spot urine sampling. These sampling times were designated as occurring in the morning (8:00 am – 12:30 pm), afternoon (1:30 – 5:30 pm), or evening (5:30 – 9:30 pm). The exact sampling time for each individual is kept confidential in NHANES, so a time was randomly assigned in our study from a uniform distribution in one of the sampling session windows. Based on NHANES data collected between 1999 and 2010, 46.7% of simulated individuals were sampled in the morning, 35.7% in the afternoon, and 17.7% in the evening (see Supplementary Table 3). Since the time of daily exposure was fixed for each simulated individual, some biomarkers for some were sampled on the 5th day after the 5th dose, while biomarkers for others were sampled on the 5th day between the 4th and 5th dose.

In summary, each of the 500 CARES individuals were assigned values for the following variables: a fixed daily dose of carbaryl which was the mean of his/her 365 CARES-simulated daily doses; a fixed time of exposure which was the mean of his/her 365 CARES-simulated time at which maximum dose occurred; a urine flow rate randomly sampled from NHANES 2009-2010; and a spot urine sampling time on the 5th day, randomly sampled from a distribution generated based on NHANES sampling sessions. Next, using the PBPK model, a corresponding urinary 1-N concentration (CARES-predicted intake doses, PBPK-predicted urinary 1-N concentrations, and model parameter values are listed in Supplementary Table 4) was predicted using these inputs (see Supplementary Figure 1, Eq. 2, red dotted histograms). These data were used in the subsequent analyses to compare three reverse dosimetry approaches. The synthetic daily intake doses were fit to a log-normal distribution for ease of comparison to population distribution

estimates generated by reverse dosimetry approaches. All simulations described in this article are summarized in Table 1.

Comparing three reverse dosimetry approaches

In the current study, three PBPK model-based reverse dosimetry approaches were evaluated: Exposure Conversion Factor (ECF), Discretized Bayesian (DBA), and Markov Chain Monte Carlo (MCMC) (Georgopoulos et al., 2009; Tan et al., 2006a). For all three methods, the only "unknown" parameter estimated from the urinary 1-N concentrations was daily intake dose. All other model parameters were kept the same for the synthetic individuals, using each method. Daily intake doses estimated from these three methods were compared to the synthetic daily intake doses.

The ECF method required a Monte Carlo (MC) simulation of the PBPK/PD model, given a unit dose of carbaryl (1 ng/kg/day) (Liao et al., 2007; Tan et al., 2006a; Tan et al., 2006b). In this analysis, however, MC randomization was not performed since the only "unknown" was the intake dose. Rather, a distribution of 500 urinary 1-N concentrations was generated by running the PBPK model using the same parameter values as those generated from the synthetic data, given a unit dose of carbaryl. Next, the reciprocal of the distribution of predicted urinary 1-N concentrations (generated from the unit dose) was calculated as the ECF distribution, in units of $\frac{\text{ng carbaryl}}{\text{kg body weight}} \Big/ \frac{\text{ng 1-N}}{\text{L urine}}$.

The ECF distribution was then convolved with the distribution of synthetic urinary 1-N concentrations to obtain an estimate of the distribution of daily intake doses of carbaryl. The ECF method is only applicable when the dose-biomarker relationship is linear. The other two methods (DBA and MCMC) do not require this assumption.

The DBA method was based on Bayes' formula (Liao et al., 2007; Tan et al., 2006a):

Equation 3: $(C_j|N) = \frac{P(N|C_j)P(C_j)}{\sum_i P(N|C_i)P(C_i)}$, for i,j= 1,2, … , T

where C is the intake dose of carbaryl, N is the urinary 1-N concentration, $P(C_j|N)$ is the probability of a carbaryl intake concentration, C_j, given an observed urinary 1-N concentration, N; $P(C_j)$ is the prior distribution for the discrete carbaryl doses, C_j; and $P(N|C_j)$ is the probability of a urinary 1-N concentration, N (predicted by a model that describes the dose-biomarker relationship), given a carbaryl dose, C_j. T is the total number of discrete carbaryl doses C_j and corresponding predicted urinary 1-N concentrations, N_j.

The ability to specify a prior distribution for exposure concentrations and to handle a non-linear dose-biomarker relationship differentiates the DBA from the ECF method. A MC simulation was run for each of the T discrete exposure doses to generate distributions of $P(N|C_j)$. The prior exposure concentrations, C_j, were selected to cover the range of possible doses and the non-linear range of the dose-biomarker relationship. This matrix for $P(N|C)$ involved rows corresponding to the number of exposure concentrations tested (T) and columns corresponding to the number of MC iterations. The matrix for $P(N|C)$ was then transformed into the posterior, $P(C|N)$ using the equation above. The transformed matrix was then multiplied by the distribution of observed biomarker concentrations, $P(N_{obs})$, to obtain the estimated distribution of carbaryl exposure for the population, P(C), according to Equation 4.

Equation 4: $P(C) = P(C|N) \times P(N_{obs})$.

In our analysis, the discrete carbaryl daily doses ranged from 10^{-2} ng/kg/day to 10^{6} ng/kg/day, with increments on a $\log_{10}$-scale by $10^{0.08}$ (T=101). This range was chosen based on the result of the ECF method, after adding a buffer of one order of magnitude. Both ECF and DBA are deterministic methods. Parameter values for each of the 500 CARES individuals were used to generate a predicted urinary 1-N concentration at a given dose. The total number of simulations

for the DBA method was 500 parameter sets × 101 unique doses = 50,500 iterations. Two priors of carbaryl intake $P(C_j)$ were used:

(1) A uniform prior (for each carbaryl intake dose C_j, the probability was the same, [10^{-2}, 10^{6}] ng/kg/day), and

(2) A biased prior (a normalized lognormal distribution with a geometric mean of 1×10^{3} ng/kg/day and a geometric standard deviation of $\sqrt{10}$, and the prior was truncated at 1 ng/kg/day and 10^{6} ng/kg/day).

The first prior was chosen to represent a non-informative case, in which only the bounds on intake doses were suggested. The second prior was chosen to represent a situation in which supporting data provided a reasonable mean exposure value; this second prior was approximated by a lognormal distribution with a large standard deviation to capture uncertainty. Even when a prior is supported, it may impose bias as it relates to the biomonitoring data used in reverse dosimetry. We wished to observe whether DBA could correct for this bias in the prior. Both ECF and DBA methods were executed using the web-based tool, PROcEED (Grulke et al., 2013) http://www.epa.gov/heasd/research/proceed.html).

The MCMC approach used an iterative application of Bayes' theorem, with the distributions regarded as continuous rather than discrete (McNally et al., 2012; Ulaszewska et al., 2012). In other words, MCMC was not confined to the range of exposure values given in the priors, in contrast to what was seen in the case of the DBA method. Specifically, $P(C|N) \propto P(C)P(N|C)$, where $P(C)$ is the prior distribution for intake doses of carbaryl, $P(N|C)$ is the likelihood function, and $P(C|N)$ is the posterior distribution of the carbaryl exposure given the observed urinary 1-N concentrations. MCMC algorithms stochastically approximate the joint-posterior distributions without having to sample the entire space and were particularly well-suited for solving non-linear inverse problems. In this study, the deterministic PBPK model was configured to run with the

population means and standard deviations for its kinetic and metabolic parameters (see Supplementary Table 1). The priors for the population mean intake were set based on a normalized lognormal distribution with a geometric mean μ_C = 100 ng/kg/day and geometric standard deviation of 200, truncated at 10^{-4} ng/kg/day and 1×10^5 ng/kg/day. The priors for the population variance were set based on a normal distribution with a mean μ_C = 100 ng/kg/day and standard deviation of 50 ng/kg/day, truncated at 10^{-4} ng/kg/day and 10^3 ng/kg/day. These priors were based on the results from the ECF and DBA methods (DBA: uniform prior) since both methods had similar distributions and a large standard deviation. The function, *N=f(C)*, represents the PBPK model for carbaryl using dose, *C* (ng/kg/day), as input and 1-N concentrations in urine, *N* (ng/L), as output. The input, "*C*", was inferred by estimating the distributions of population mean and variance (Bois, 2000) using AcslX (The AEgis Technologies Groups, Inc., Huntsville, AL). It is a common practice to remove the burn-in from the resulting chains, and thus, the first 7,000 iterations were removed in our analysis. Fifty sets of mean and variance were selected from the MCMC output chains to generate 50 possible distributions of "*C*", and then 500 values were randomly selected from each of the 50 distributions to obtain 25,000 "*C*" possibilities, which contributed to the final estimates of the distribution of "*C*".

Evaluating the value of information in exposure reconstruction

The approach presented above allowed us to evaluate the efficiency of different reverse dosimetry methods in reconstruction of daily intake doses when these doses were the only unknown (referred to as "all parameters known"). The impact of missing information in exposure reconstruction was evaluated by (1) setting all parameter values to their means, (2) setting individual parameter values to either (2) their *known* value, or (3) a random value from population distributions supported by literature. The parameters we tested in this analysis were: (1) elapsed time between the final

dose and urine biomarker sampling (potentially measurable), (2) urine flow rate (potentially measurable), and (3) urinary elimination rate of 1-N and its metabolites (the most sensitive parameter from the local sensitivity analysis, but not directly measurable in humans).

A common practice for reconstructing daily intake doses based on real-world biomarker data involves setting model parameters to their respective means, which is assumed to result in reasonable estimates in the absence of measured data. Thus, in this first analysis (Case 1), certain model parameters of interest were replaced with their respective means.

Case 1 (Means): All of the parameters being tested were set to their means.

(1) The elapsed time between the final dose and urine sampling was set to -0.865 hours (after the fourth day's dose, but just before the fifth day's dose). This was the mean from our 500 synthetic individuals.
(2) The urine flow rate was set to 0.6526 mL/min based on the mean of NHANES 2009-2010 (CDC, 2011) .
(3) The most sensitive PBPK parameter, the urinary elimination rate was set to its mean, 0.2/h/kg$^{-1/4}$ (Yoon et al., 2012).

The MCMC method, with the same priors as described above, was used to reconstruct daily intake doses to investigate the impact of using population means for all model parameters on reconstructing population intakes (Case 1).

In the next two components of the analysis (Cases 2 and 3), all parameters were set to their mean values, except for the three parameters mentioned above (e.g., elapsed time between dose and sampling, urine flow rate, and urine elimination rate). Rather than using the means for all three parameters as with case 1, two parameters were set to their means one at a time, while the third was altered as described below for each individual case:

Case 2 (Default): The parameter being tested was assigned independently to the synthetic individual values used to generate the urinary 1-N concentrations. This case corresponds to a situation in which measurements of the elapsed time, urine flow rate, or urinary elimination rate are collected as part of a biomonitoring study.

Case 3 (Random): The parameter being tested was randomly selected from a distribution:

(1) A normal distribution for elapsed time between the final dose and urine sampling, $t_{elapsed} \sim N(-.86523, 6)$, in hours. This distribution was obtained from the synthetic individuals, with a wider standard deviation to account for uncertainty.
(2) A lognormal distribution for the urine flow rate with a geometric mean of 0.6526 mL/min and a geometric standard deviation of $\sqrt{10}$. This distribution was obtained from NHANES 2009-2010 (CDC, 2011) , with a wider standard deviation to account for uncertainty.
(3) A lognormal distribution for the urinary elimination rate of 1-N, with a geometric mean of $0.2/h/kg^{-1/4}$ and a geometric standard deviation of 1. This distribution was obtained from the literature based on animal values (Knaak, 1968; May et al., 1992; Yoon et al., 2012), with a wider standard deviation to account for uncertainty.

Case 3 is similar to setting all parameter values to their respective means (Case 1); however, the parameter distribution was inferred from other information in addition to the mean values. For example, the exact time of exposure events may not be recorded in the biomonitoring study, but the general time frame for sampling collection might be known (e.g., between 9 am and 5 pm). Or, urine flow rate may be estimated from a carefully measured urine void volume and self-reported time between voids, which were subject to uncertainty inherent in human recalls. Or, a distribution of urinary elimination rate of 1-N may be obtained from the literature since this parameter is only measurable in animals.

As described earlier, the burn-in of 7,000 iterations were removed in all cases, except when examining the influence of urinary elimination rate. Because the Markov chain converged faster when updating urinary elimination rate, only the first 3,000 iterations were removed. These six additional trials (from Cases 2 and 3: 3 parameters $\times$ 2 cases) aided in the evaluation of the value of incorporating additional information for specific parameters, using the MCMC method. A summary of the different simulations and analyses described above is given in Table 1.

A Welch t-test was conducted to determine if the means of each of the MCMC posterior distributions of intakes were significant different than the mean of the CARES-synthetic intake distribution. This test was repeated for each of the MCMC simulations.

RESULTS

The CARES model was used to estimate daily carbaryl intake doses for 500 simulated individuals, which were then fit to a log-normal distribution (Figure 1). These synthetic intake doses were then compared against other "reconstructed doses" (Tables 2 and 3). The CARES-synthetic geometric mean was 70 ng/kg/day, and the geometric SD was 4.1 (Table 2). Comparison of the population distribution estimates of carbaryl daily intake doses from the three reverse dosimetry methods with the distribution of the CARES- synthetic daily intake doses showed that all three methods were reasonably good at estimating the mean of the distribution (Table 2). The estimated geometric mean daily intake was 97, 100, 251, and 92 ng/kg/day for the ECF, the DBA (uniform prior), the DBA (biased prior) and MCMC, respectively (Table 2). The mean intake doses estimated by the three reverse dosimetry methods (ECF, DBA: uniform prior, and MCMC) were more similar to each other than to the mean CARES-simulated dose, and all three methods overestimated the mean intake dose (Table 2). The ECF and the DBA (both priors) methods provided similar estimates of the population SD, but both these estimates were significantly larger (about 200 times) than the CARES-synthetic SD. On the other hand, the MCMC-estimated geometric SD was 4.5, which was fairly similar to the CARES-synthetic geometric SD. Thus, out of the three reverse dosimetry methods, MCMC performed the best in our dose reconstruction analysis.

Comparing the posterior distributions obtained from two different priors in the DBA method, the posterior mean updated from the uniform prior was more similar to the population geometric mean of the CARES-synthetic intake doses (Figure 2a, black line vs blue dashed-dotted line). While the posterior mean updated from the non-uniform (biased) prior remained biased (Fig 2b. black line vs blue dashed-dotted line), the posterior mean was improved compared to its prior (Fig 2b, black line vs red dotted line). Additionally, the posterior distribution updated from the non-uniform prior was tighter and more precise, though less accurate, than that updated from the uniform prior (Figure 2).

Next, the impact of missing information was evaluated. The MCMC analysis from the method comparison was included for purposes of comparison. The distribution generated assuming "all parameter values are known" had a GM of 92 ng/kg/day, and a GSD of 4.5; while the distribution generated by "setting all parameter values to their respective means" (case 1) had a geometric mean of 47 ng/kg/day, and the geometric SD was 0.8 (Table 3).

For five of the six MCMC trials, the inferred GM (ranging from 352 to 690 ng/kg/day) overestimated the CARES-synthetic GM (70 ng/kg/day) by one order of magnitude (Table 3). The only exception was for the case in which urine flow rates were randomly selected from a distribution (Table 3, "Urine Flow Rate, MCMC-random"). In this case, the geometric mean, 45,968 ng/kg/day, was three orders of magnitude greater than that of the CARES-synthetic daily intake doses (Table 3). All six MCMC trials overestimated the geometric SD (ranging from 59 to 30,055). Again, the "Urine Flow Rate, MCMC-random" case resulted in the largest estimate of the geometric SD (Table 3).

Intake doses in the "Urinary Elimination Rate, MCMC-default" and the Urinary Elimination Rate, MCMC-random" cases were similar to each other, with slightly less error in the MCMC-random case (Table 3). Estimated intake doses for the elapsed time and urine flow rate, MCMC-random cases showed a larger geometric mean/SD than did intake doses in the MCMC-default cases for both parameters (Table 3). However, the performance of the dose reconstruction was extreme in both cases for urine flow rate. MCMC-default (Case 2: urine flow rate) performed the best among the six cases, while the MCMC-random (Case 3: urine flow rate) performed the worst among the six cases (Table 3). This finding indicates that knowledge of urine flow rate is critical when attempting to reconstruct doses based on urine metabolites of short half-life chemicals.

A Welch's t-test revealed that the means of each of seven out of the eight MCMC distributions were significantly different (0.05 level) from the mean of the CARES-synthetic distribution (Table

3). When all parameters were set to their means (Case 1) the mean of the MCMC distribution was not significantly different than the mean of the CARES-synthetic distribution.

DISCUSSION

In their publication "*Exposure Science in the 21st Century*", the National Research Council reported that biomarker data "will be essential for evaluating the efficacy of exposure reduction policies, and for prioritizing and assessing chemical risks" (NRC, 2012). One way to achieve these goals is to convert biomarker data to intake doses for comparison to an established exposure guidance value. Exposure guidance values are usually determined through animal toxicity studies, in which administered target tissue doses are known and measurable. In humans, however, most target organs cannot be examined, and often only biomarkers in accessible media can be collected. Due to the difficulty in directly associating biomarker measurements with target tissue doses, the common approach for biomarker use in risk assessment is conversion of its concentration to an exposure level. One basic assumption that is often ignored with this approach is that the biomarker should have a strong, direct correlation with intake doses (LaKind et al., 2014). In cases where the biomarker is a poor surrogate of intake doses, which often occurs for short half-life chemicals, these biomarker measurements are only suitable for trend analysis (e.g. do biomarker concentrations change with time?) or comparison among different groups (e.g. male/female).

In the current study, the ability of three different reverse dosimetry approaches to reconstruct intake doses was investigated using model-simulated data. Corresponding intake doses, physiological measurements, and pharmacokinetic data are rarely collected in conjunction with biomarker measurements. Thus, the most viable approach is to generate "unmeasured" data using models (Georgopoulos et al., 2009; Phillips et al., 2014a; Phillips et al., 2014b). For example, Georgopoulos et al., (2009) also compared the performance of the ECF and the DBA models using actual biomarker data with known exposure data or "synthetically augmented" data (i.e., missing information was filled using randomly sampled values from distributions) and found that reconstruction using the synthetic data better facilitated the evaluation of reverse dosimetry methods and characterization of the value of additional information.

In our study, comparison of the three reverse dosimetry approaches in reconstruction of intake doses based on urinary biomarkers suggests that MCMC exhibited the best capability at identifying the population variance. The use of the MCMC, however, requires increased computational resources compared to the other two methods explored in this study. Seventy-two hours was necessary for the completion of a hierarchical analysis on a quad-core 2.2 GHz i7 MacBook, while only minutes were necessary for completion of the ECF and the DBA models using PROcEED (Grulke et al., 2013). Further computational/runtime improvements may be possible if the population size was reduced from 500 individuals, as the number of simulation runs required per MCMC iteration scales with the number of individuals. Such reduction, however, is unlikely to be realistic when interpreting biomarker results from large-scale studies, such as NHANES.

Other reverse dosimetry approaches that are not evaluated in the current study, such as optimization or trial-and-error approach (Mosquin et al., 2009; Roy and Georgopoulos, 1998) and "multiplier" (e.g., fraction of total dose in urine) can back-calculate intake doses from biomarker data (Lakind and Naiman, 2008; Lorber et al., 2011; Payne-Sturges et al., 2009). The performance of the "multiplier" approach depends solely upon the accuracy of the "multiplier", and the performance of the optimization approach is highly related to the optimization routine selected. Bayesian approaches, such as the DBA method examined in the current study, can also be implemented as an optimization scheme.

Given that the MCMC method exhibited the ability to closely infer the population mean and variance of synthetic daily carbaryl intakes simulated from CARES, this modeling approach was also used to evaluate the impact on reconstructed doses from uncertainty in specific parameters. Case 1, which is the only MCMC case that estimated a population mean not significantly different from the CARES-synthetic mean, is analogous to representing the entire population using an "average individual". Thus, the estimated average intake dose adequately reflects the CARES average synthetic intake dose. This finding is consistent with the general agreement that the

central tendency of the distribution of biomarker concentrations is to reflect long-term average exposures in a population (Aylward et al., 2012; Pleil and Sobus, 2013; Rao et al., 2012). Since the only variability in this case came from urinary 1-N (biomarker) concentrations (all parameters were set to their means), the estimated SD was the smallest among all cases.

The MCMC case in which all parameter values were “known” and independently assigned for individuals would have been expected to provide the best estimates of intake dose. While the estimated mean from this case slightly overestimated the CARES-synthetic mean, this MCMC case did provide the best estimate of the overall distribution (Table 3). In addition to the variability in urinary 1-N concentrations, this MCMC case also included the variability in PBPK parameters, urine flow rate, and time of urine sampling. The inclusion of these parameter values provided sufficient information for updating the intake estimates. As a result, this MCMC case was able to predict a similar variance as the CARES-synthetic distribution.

In our simulation study, the value for each parameter was known, which made the MCMC-default case possible (MCMC from method comparison, and Case 2). In real life, however, it is not often feasible to collect a specific piece of information from each individual in a population. In some cases, certain data (e.g., time between urine voids) can be collected as part of the biomonitoring study if the study designers are aware of these parameters’ importance. Often, information is available only at the population level, and the value of an unmeasured parameter may be estimated based on the central tendency of a distribution (set all parameter values to their means) or the entire distribution for the population (randomly select parameter values from distributions).

Out of the three parameters selected for evaluating the impact of missing information in this study, urine flow rate was the most influential on the performance of the dose reconstruction. Dose reconstruction using MCMC requires the comparison of model predictions to measured biomarker data, in this case the concentration of 1-N in urine, to update the intake dose estimates. Urinary 1-

N concentration is calculated by dividing the PBPK model-predicted mass flow rate of 1-N into the urine (ng/h), *r(t)*, by the urinary flow rate, *fr* (see the flow rate calculation, Eq. 2). In other words, a single urinary 1-N concentration may be calculated from infinite combinations of model-predicted 1-N excretion rates and urine flow rates (i.e., no unique solution). As a result, MCMC was unable to estimate a reasonable intake distribution when urine flow rate was allowed to vary ("Urine Flow Rate, MCMC-random", Case 3, Table 3). Alternatively, when *fr* was assigned using each individual's value ("Urine Flow Rate, MCMC-default", Case 2, Table 3), the reconstructed mean intake dose was the closest (of the six presented cases) to the CARES-simulated mean despite a significant difference still existing between the two means. Another study that examined contributors to biomarker variability, assuming a single dose, also identified variability in urine flow rate as a major influence compared to variability in other physiological or pharmacokinetic parameters (Phillips et al., 2014a). In 2009-2010, urine flow rates began being included in the NHANES sampling data set (CDC, 2011). To accomplish this, volume of urine was collected and participants were asked to recall the time of their last void. Urine flow rate was obtained by dividing urine volume by the time between voids. This is a promising step towards fixing data gaps in the use of biomarker data to understand exposure, although it should be noted that uncertainties in recollection of void times could still lead to data inaccuracies.

The second circumstance in which it proved difficult to predict a parameter value was demonstrated through the uncertainty in the parameter investigated: elapsed time between the final dose and the time of urine sampling. For short half-life chemicals, a larger intake dose with longer elapsed time and a smaller dose with shorter elapsed time may result in the same biomarker concentrations. Comparing between the two MCMC cases, "Elapsed Time, MCMC-default" and "Elapsed Time, MCMC-random", (Cases 2 and 3, respectively, Table 3), the difficulty of accurately estimating the magnitude of intake doses was demonstrated when the elapsed time between the final exposure dose and urine sampling is not recorded in a biomonitoring study

(Case 3: MCMC-random). Both elapsed time and urine flow rate (or, alternatively, the void volume and time between voids) are data that can be collected easily. The accuracy of these values can be greatly improved when the study managers are made aware of importance of recording these data rather than having the participants recall the information.

The third parameter investigated in our study was the urine elimination rate. The estimated intake doses were similar whether this parameter was set to known values ("Urine Elimination Rate, MCMC-default", Case 2) or assigned randomly from a distribution ("Urine Elimination Rate, MCMC-random", Case 3) (Table 3). In other words, randomly sampling from a distribution was appropriate enough to represent the urine elimination rates for individuals. This finding is reassuring because urine elimination rate is not measurable in humans, and its distribution may be obtained from animal studies.

We generally found that all of the six cases (MC-default and MCMC-random) overestimated the population mean and variance of the carbaryl intake doses compared to the MCMC case in which all parameters were set to their respective means. A likely explanation for this overestimation is that urine flow rates and urine elimination rates are log-normally distributed. This implies that values much larger than the geometric mean were included in the MCMC analysis, resulting in larger estimations of intake doses. While the elapsed time between the final dose and urine sampling was assumed normally distributed, we did increase the SD of the distribution to account for uncertainty. As a result, much longer elapsed times were included in the MCMC analysis, also resulting in higher estimated intake doses.

CONCLUSIONS

In conclusion, our study has illustrated the trade-offs between using non-iterative methods for exposure reconstruction (e.g. ECF, and DBA) vs. iterative methods (e.g. MCMC), as well as the impact of uncertainty in specific model parameters in exposure reconstruction methods. This study has demonstrated the importance of including measurements for urine flow rate (or volume of void, and time between voids) and elapsed time between last dose and urine sampling as part of the biomarker sampling collection. Including these measurements in biomonitoring studies will facilitate more accurate exposure reconstruction, allowing for interpreting biomarker data in a risk context. Without these measurements, the uncertainty surrounding exposure estimates may dramatically limit the interpretation of biomarker results. If critical data gaps can be resolved, especially for unidentifiable model parameters, exposure reconstruction methods (e.g. MCMC) can be utilized to better predict population-level intake doses from large biomonitoring studies.

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Table 1. Descriptions of simulations presented in this article.

Simulation	Description	Exposure Scenarios	Model Outputs
Sensitivity Analysis	Determine the most sensitive PBPK model parameters.	One dose per day at 2:42pm. 3 doses tested corresponding to 5th, 50th, and 95th percentile of	Normalized sensitivity coefficients for the PBPK model parameters.

		CARES daily doses. 3 elapsed times between daily dose and urine sampling were tested 1, 4, and 12 hours. All other parameters were set to their means.	
Computation of biomarker concentration from model output	Two different methods for calculating biomarker concentration were compared (volume, and flow rate calculations)	Random week of intermittent exposures from food and water as specified in the CARES model for 500 virtual individuals. All other parameters were set to the synthetic individual values.	Single spot urinary 1-N concentrations based on both volume and flow rate calculations (500 each).
Synthetic intakes and biomarkers (paired data)	Model-generated corresponding dose-biomarker dataset for exposure reconstruction.	One dose per day for 5 days, dose is the mean of 365 daily doses, given at the median time when the maximum dose occurred over 365 days. Flow rate calculation was used. Parameters were set to synthetic individual values.	Paired daily intakes and corresponding spot urinary 1-N concentrations (biomarker) for 500 synthetic individuals.
Simulation for the ECF method	The dose-biomarker relationship for converting biomarker concentrations to doses in the ECF method.	1 ng/kg/day for 5 days given at the median time when the max dose occurred over 365 days. All other parameters were the same as the synthetic individuals (n=500).	500 single spot urinary 1-N concentrations based on the flow rate calculation compared to synthetic biomarkers, to estimate the intake distribution.
Simulation for the DBA method	The dose-biomarker relationship for converting biomarker concentrations to doses in the DBA method.	Intake doses ranged from 10^{-2} to 10^{6} ng/kg/day, incrementing on a $\log_{10}$-scale by $10^{0.08}$(N=101), each dose was repeated for 5 days. All other parameters were the same as the synthetic individuals (n=500).	500 single spot urinary 1-N concentrations based on the flow rate calculation (for each of the 101 intakes), which were compared to synthetic biomarkers, to estimate the intake distribution.
MCMC for Method Comparison, all parameters known.	In a single MCMC iteration: For each updated prior of exposures, biomarkers are predicted for the population and compared to the synthetic biomarkers.	One dose per day for 5 days, given at the median time when the max dose occurred over 365 days. The distribution of exposure dose is updated at each step of the MCMC. All other parameters were the same	Single spot urinary 1-N concentrations (based on the flow rate calculation, n=500 per MCMC iteration), compared to synthetic biomarkers to

		as the synthetic individuals (n=500)	estimate posterior distribution of intakes.
MCMC, for testing different parameters of interest: Case 1, All parameters set at their mean.	For each updated prior of exposures, biomarkers are predicted for the population and compared to the synthetic biomarkers.	One dose per day for 5 days, given at the median time when the max dose occurred over 365 days. The distribution of exposure dose is updated at each step of the MCMC. All parameters, including the time of sampling, were set to their respective means.	500 single spot urinary 1-N concentrations (based on the flow rate calculation), per MCMC iteration. These biomarker concentrations were compared to the synthetic biomarkers to estimate posterior intake distribution.
MCMC, for testing different parameters of interest: Case 2 (Default)	Same as above.	Same as above, except that the parameter of interest was set to known individual values, and all other parameters were set to their mean.	Same as above.
MCMC, for testing different parameters of interest: Case 3 (Random)	Same as above.	Same as above, except that the parameter of interest was set to randomly selected individual values from a distribution, and all other parameters were set to their mean.	Same as above.

TABLE 2. Comparing the geometric means and geometric standard deviations for carbaryl intake dose estimated from CARES against those reconstructed using the Exposure Conversion Factor (ECF), the Discretized Bayesian Approach (DBA) using the both the uniform prior and the biased prior, and Markov Chain Monte Carlo (MCMC) methods.

	Geo. Mean (ng/kg/day)	Geo. Std. Dev.
CARES-synthetic daily intake	70	4.1
ECF-reconstructed daily intake	97	787
DBA-reconstructed daily intake (uniform)	100	795
DBA-reconstructed daily intake (biased)	251	663
MCMC-reconstructed daily intake	92	4.5

Table 3. Comparing the geometric means and geometric standard deviations for carbaryl intake dose estimated from CARES against those reconstructed from Markov Chain Monte Carlo (MCMC) methods assuming either all parameters were known (from method comparison analysis) or set to their respective means (Case 1). Six additional MCMC trials were also included for comparison: setting elapsed time between the last dose and urine sampling (Elapsed Time), urine flow rate, or urinary elimination rate to either the values used to generate the 1-naphthol (1-N) concentrations in urine (Case 2, default), or to values generated from random sampling from a distribution (Case 3, random).

	Geo. Mean (ng/kg/day)	Geo. Std. Dev.
CARES-synthetic daily intake	70	4.1
MCMC – all parameters known	92*	4.5
MCMC – all parameters set at their means	47	0.8
Elapsed Time		
MCMC – default	393*	59
MCMC – random	690*	116
Urine Flow Rate		
MCMC – default	352*	67
MCMC – random	45,968*	30,055
Urinary Elimination Rate		
MCMC – default	508*	93
MCMC – random	507*	83

* indicates that the mean is significantly different than the mean of the CARES-synthetic distribution, using a Welch t-test with a 0.05 significance level.

Figure Legend

Figure 1. Probability density of carbaryl intake (ng/kg/day) for 500 simulated individuals. The x-axis has been $\log_{10}$ transformed. The black boxes show the histogram of the carbaryl intake doses generated using the CARES model that served as the input data for the PBPK simulation. The blue dashed-dotted line shows the lognormal distribution that was fitted to the carbaryl intake data.

Figure 2. Effect of the prior distribution on the results of Discretized Bayesian Analysis (DBA) for reconstructing intakes of carbaryl (ng/kg/day). (A) Uniform DBA prior (red dashed line); the corresponding estimate from DBA (solid black line); simulation input (from Figure 1, dashed-dotted blue line) shown for comparison. (B) Biased DBA prior: lognormal distribution (geometric mean: 10^3 ng/kg/day; geometric standard deviation: $\sqrt{10}$) truncated at 10^{-2} (lower bound) and 10^6 (upper bound) (red dashed line); the corresponding estimate from DBA (solid black line); simulation input (from Figure 1, dashed-dotted blue line) shown for comparison.

Figure 1

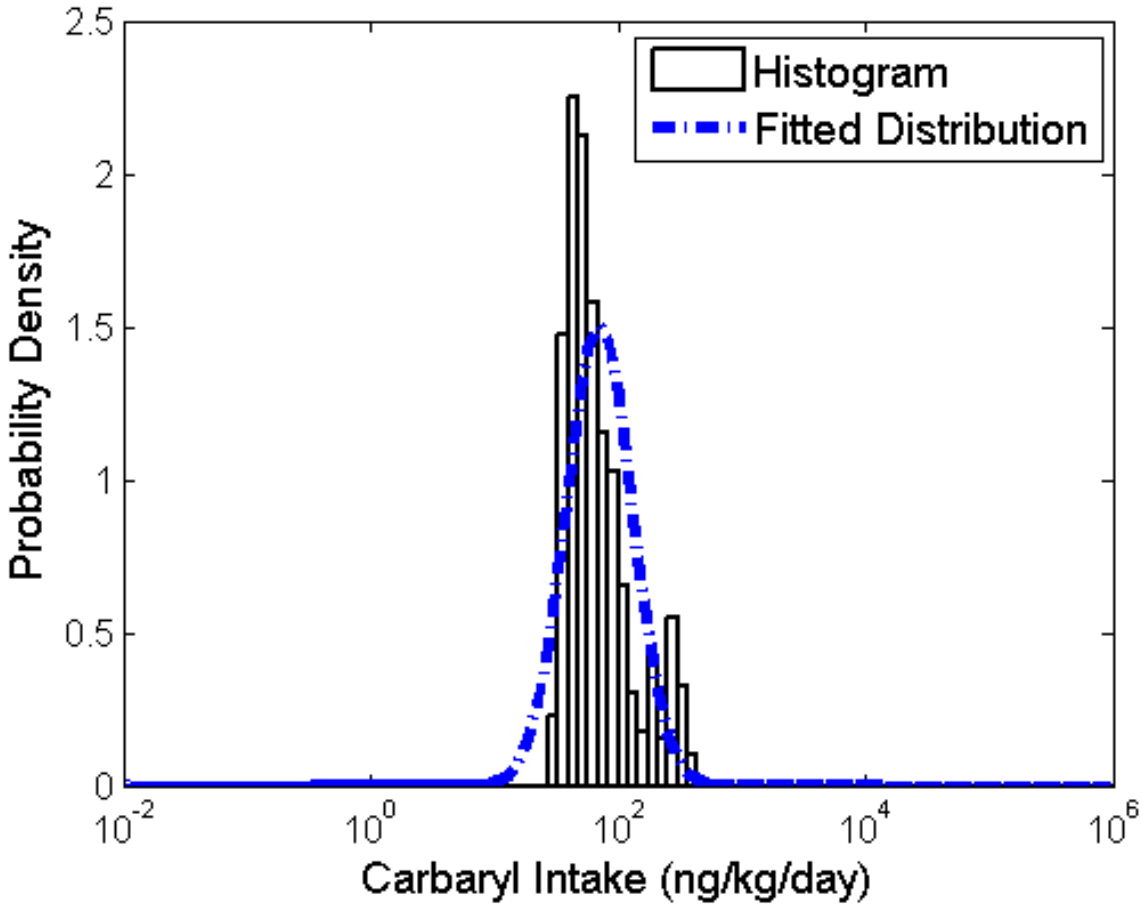
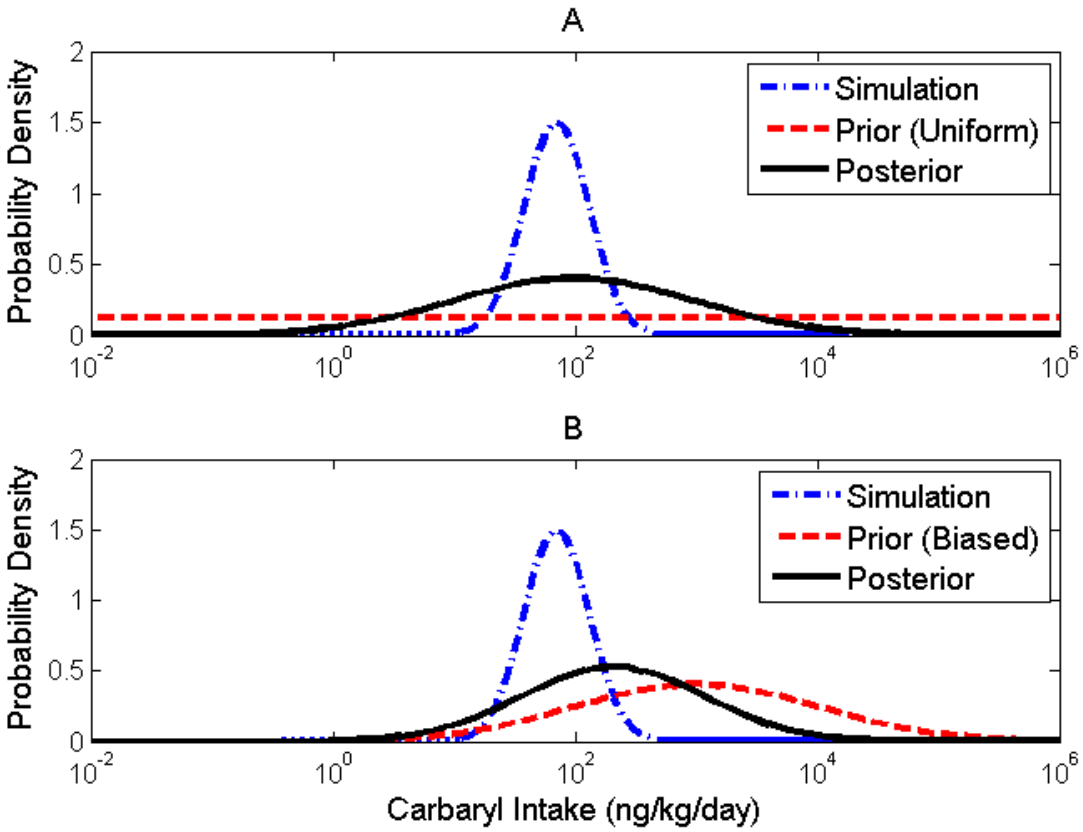

Histogram
Fitted Distribution
Probability Density
2.5
2
1.5
1
0.5
0
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
Carbaryl Intake (ng/kg/day)

Figure 2


A
Probability Density
2
1.5
1
0.5
0
Simulation
Prior (Uniform)
Posterior
10^{-2}
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10^{2}
10^{4}
10^{6}
B
Probability Density
2
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Simulation
Prior (Biased)
Posterior
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
Carbaryl Intake (ng/kg/day)