A New Method for Generating Distributions of Biomonitoring Equivalents to Support Exposure Assessment and Prioritization

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ABSTRACT

Biomonitoring data are now available for hundreds of chemicals through state and national health surveys. Exposure guidance values also exist for many of these chemicals. Several methods are frequently used to evaluate biomarker data with respect to a guidance value. The "biomonitoring equivalent" (BE) approach estimates a single biomarker concentration (called the BE) that corresponds to a guidance value (e.g., Maximum Contaminant Level, Reference Dose, etc.), which can then be compared with measured biomarker data. The resulting "hazard quotient" estimates (HQ = biomarker concentration/BE) can then be used to prioritize chemicals for follow-up examinations. This approach is used exclusively for population-level assessments, and works best when the central tendency of measurement data is considered. Complementary approaches are therefore needed for assessing individual biomarker levels, particularly those that fall within the upper percentiles of measurement distributions. In this case study, probabilistic models were first used to generate distributions of BEs for perchlorate based on the point-of-departure (POD) of 7 μg/kg/day. These distributions reflect possible biomarker concentrations in a hypothetical population where all individuals are exposed at the POD. A statistical analysis was then performed to evaluate urinary perchlorate measurements from adults in the 2001-2002 National Health and Nutrition Examination Survey (NHANES). Each NHANES adult was assumed to have experienced repeated exposure at the POD, and their biomarker concentration was interpreted probabilistically with respect to a BE distribution. The HQ based on the geometric mean (GM) urinary perchlorate concentration was estimated to be much lower than unity (HQ ≈ 0.07). This result suggests that the *average* NHANES adult was exposed to perchlorate at a level well below the POD. Regarding individuals, at least a 99.8% probability was calculated for all but two NHANES adults that a

higher biomarker concentration would have been observed compared to what was actually measured if the daily dietary exposure had been at the POD. This is strong evidence that *individual* perchlorate exposures in the 2001-2002 NHANES adult population were likely well below the POD. This case study demonstrates that the "stochastic BE approach" provides useful quantitative metrics, in addition to HQ estimates, for comparison across chemicals. This methodology should be considered when evaluating biomarker measurements against exposure guidance values, and when examining chemicals that have been identified as needing follow-up investigation based on existing HQ estimates.

KEYWORDS

Biomarker; biomonitoring; Biomonitoring Equivalent; prioritization; NHANES; PBPK; perchlorate; urine

ABBREVIATIONS

ADME: absorption, distribution, metabolism, elimination; ATUS: American Time Use Survey; BE: biomonitoring equivalent; BE_{POD}: biomonitoring equivalent corresponding to the point of departure; BW: body weight; CDC: Centers for Disease Control and Prevention; GM: geometric mean; GSD: geometric standard deviation; HQ: hazard quotient; HQ_{GM}: hazard quotient corresponding to the geometric mean; HQ_{95}: hazard quotient corresponding to the 95^{th} percentile; mpd: meals per day; NHANES: National Health and Nutrition Examination Survey; NOAEL: no observed adverse effect level; NSC: normalized sensitivity coefficient; PBPK: physiologically-based pharmacokinetic; POD: point of departure; UO: urine output (L/hr)

1. INTRODUCTION

Humans are exposed to thousands of distinct chemicals every day from both natural and man-made sources (USEPA 2013c). Understanding the impacts of these exposures on human health requires accurate and precise exposure estimation. Conventional methods for exposure estimation integrate environmental measurements, records of human time/location activities, and other exposure factors (e.g., hand-to-mouth frequency) (USEPA 1992). Alternative strategies now utilize chemical biomarker measurements (USNRC 2006). Biomarkers of exposure are generally parent chemicals or their metabolite(s) measured in biological media (Fields and Horstman 1979). These measurements provide direct evidence of human exposure to a chemical. Given the availability of these data in nationwide exposure and health surveys (e.g., the Centers for Disease Control and Prevention's [CDC] National Health and Nutrition Examination Survey [NHANES]), there is a move to make use of biomarker measurements to support exposure and risk assessments.

Several approaches exist for evaluating exposures and/or health risks using biomarker data. The "Biomonitoring Equivalent" (BE) approach, developed by Hays and colleagues, is a popular screening method for comparing biomarker data to exposure guidance values (Hays et al. 2007). This approach uses pharmacokinetic (PK) models or analytical expressions to predict a steady state or average biomarker concentration given exposure at an existing guidance value. The predicted biomarker concentration (i.e., the BE) is then compared to biomarker measurements from a population of interest to estimate a hazard quotient (HQ), where HQ = [Biomarker Concentration]/BE. These HQs can be compared across chemicals to identify those that are of higher concern with respect to human health risk.

To date, the BE approach has been used to interpret biomonitoring data for approximately 100 chemicals in a wide range of classes, such as dioxins (Aylward et al. 2008), phthalates (Aylward et al. 2009), and heavy metals (Hays et al. 2010). In a recent article by Aylward and colleagues (Aylward et al. 2013), HQs were calculated based on geometric mean (HQ_{GM}) and 95th percentile (HQ_{95}) estimates for a subset of analytes measured as part of the NHANES. These calculated HQs were used to prioritize chemicals; a small number had HQ_{GM} near unity. The number of chemicals with HQ_{95} near unity increased, but the importance of HQ_{95} is difficult to interpret.

There is general agreement that the central tendency of a distribution of biomarker measurements reflects longer-term average exposures in the sample population (Aylward et al. 2012, Pleil and Sobus 2013). Thus, HQ estimates based on GM levels provide insight into these longer-term average exposures, where estimates near unity suggest that population-wide exposure is likely to be near the guidance level. However, HQ estimates based on distribution tails are much more difficult to interpret. For distributions of short-lived biomarkers, it is difficult and sometimes impossible to tell whether very high levels reflect elevated acute exposures, chronic exposures, or a combination of factors that are independent of exposure magnitude (e.g., the timing of exposure with respect to sample collection). This limitation has important implications for interpreting biomarker measurements within a risk context since regulatory agencies establish tolerable limits based on chronic exposure. Thus, new strategies are needed for interpreting biomarker distribution tails with respect to BEs and other biomarker-based screening values.

The current study presents a stochastic approach for estimating a distribution of BEs that takes into account both exposure and PK variability. A statistical methodology is also presented

for interpreting tails of a measured biomarker distribution with respect to the estimated BE distribution. These techniques are illustrated using perchlorate as a case study. Perchlorate was chosen because of the abundance of relevant exposure and biomarker data, as well as the existence of a physiologically-based pharmacokinetic (PBPK) model that describes the dose-biomarker relationship. The stochastic approach presented in this article may be used to supplement existing HQ estimates and to facilitate the quantitative interpretation of human biomonitoring data.

2. METHODS

2.1 Stochastic BE Approach

The new stochastic BE approach is based on the original BE approach developed by Hays and colleagues (Hays et al. 2007). Similarities and key differences between approaches are illustrated in Figure 1 and are discussed in detail in the following subsections. Throughout the article, perchlorate is used as a case study chemical to demonstrate the stochastic approach. Perchlorate is a well-studied chemical that is used in rocket fuel, explosives, and fireworks (Motzer 2001). It also originates from natural sources, and is a byproduct of some water disinfection processes (Rao et al. 2012). Dietary ingestion is considered the major route of environmental exposure to perchlorate (Huber et al. 2011, Mendez et al. 2010, Murray et al. 2008), and is therefore the only exposure route considered in this analysis.

2.1.1 Exposure guidance value

Generally an existing exposure guidance value is considered the starting point for BE derivation (Aylward et al. 2009). In some cases, the starting point is a point of departure (POD), which is the dose-response point that marks the beginning of a low-dose extrapolation (USEPA 2014). An example of a POD is a no observed adverse effect level (NOAEL), which is the highest dose tested that does not produce an adverse effect (USEPA 2013a, USNRC 2005). When a POD is based on studies of laboratory animals, an uncertainty factor (e.g., 10×) is used to adjust for interspecies differences prior to the derivation of a BE. For our case study of perchlorate, the POD is a NOAEL of 7 μg/kg/day (USNRC 2005) that was determined from human studies (Greer et al. 2002). Therefore, no interspecies uncertainty factor is required, and the human POD was chosen to be the starting point of our case study.

2.1.2 Exposure model

Once a guidance value is selected as a starting point, it is incorporated into the BE calculation as the exposure concentration. As shown in Figure 1, the original approach uses a simplified scenario of continuous steady state exposure, whereas the stochastic approach uses a probabilistic exposure model to simulate real-life scenarios. In this study, multiple perchlorate dietary exposure scenarios (one or three meals per day [mpd] at different meal times) were simulated. One mpd exposures were simulated with the total daily dose (7 μg/kg/day) given at 7:00 am, noon, or a randomized time based on a distribution of meal times gathered from the American Time Use Survey (Figure S2). Three mpd simulations were simulated based on a fraction of the total daily dose (7 μg/kg/day) given at 7:00 am, noon, and 5 pm, or at three randomized times based on the ATUS. For these 3 mpd cases with randomized meal times, simulated days were first segmented into breakfast (midnight – 10:30 am), lunch (10:30 am – 3:00 pm), and dinner (3:00 pm – midnight) time frames. Each meal time was then randomized using the ATUS by choosing one meal from within each time frame with no meal being allowed to take place less than one hour after the previous meal. The daily dose was split across the three meals in one of the two ways: (1) each meal was one third of the total daily dose; or (2) each dose was set to a fraction using randomization that was no less than 10% or more than 80% of the total daily dose, while constraining the sum of the three meals to add up to 100%.

2.1.3 Dosimetry model

Dosimetry models, including PBPK models and analytical expressions, can be used to predict biomarker concentrations corresponding to known external exposures. Whereas the

original BE approach uses fixed (e.g., at the population median) model parameter values to simulate biomarker concentrations, the stochastic BE approach samples from distributions to get values of key model parameters. For this case study, an existing PBPK model for perchlorate was used to incorporate physiologic and PK parameter variability into BE estimates (Merrill et al. 2005, USEPA 2009). A local sensitivity analysis was performed on the PBPK model to identify sensitive parameters for the selected response variables, which were the amount of perchlorate in the urinary compartment (μg) and the mass flow rate of perchlorate into the urinary compartment (μg/hr). Any parameter with a median normalized sensitivity coefficient (NSC) during the sampling period greater than 0.1 was considered sensitive (Peters 2012). For each sensitive parameter, the shape (i.e., normal or lognormal), central tendency (i.e., mean or geometric mean), and coefficient of variation were obtained from the literature (see supplementary material for full details of the distributions of PBPK parameters, Table S1). In cases where a coefficient of variation was not reported, it was assigned a value of 50%.

2.1.4 Urine output model

Urinary biomarker concentrations are typically measured and reported in units of concentration (mass of chemical / volume of urine) or creatinine-adjusted concentration (mass of chemical / mass of creatinine). As previously mentioned, dosimetry models predict the amount of chemical in a urinary compartment (μg), and mass flow rate into that compartment (μg/hr). Thus, the volume of urine (L) or urine output rate (L/hr) is needed to convert model predicted values into concentration estimates (μg/L), and the mass of excreted creatinine (mg) or creatinine excretion rate (mg/hr) is needed to convert to creatinine-adjusted concentrations (μg/mg). The original BE approach assumes constant rates of urine output or creatinine excretion for

estimating steady state or time-averaged biomarker concentrations. The stochastic BE approach samples from distributions of these values, since they are known to vary within and between individuals (Fortin et al. 2008). For this case study, urine output and creatinine excretion data subsets were selected from the 2009-2010 NHANES dataset (the only NHANES dataset in which these values are reported). Specifically, data subsets were selected based on participants' gender, age, body weight, and lab session. Lab session was the time block in which urine sampling took place. It was reported as one of three sessions: morning (8:00 am - 12:30 pm), afternoon (1:30 - 5:30 pm), or evening (5:30 - 9:30 pm). Since urine output and creatinine excretion can be influenced by the four factors listed above (Zewdie et al. 2010), measurements were binned as follows: gender (male or female); age (20-45 or 46+); weight (≤79.12 kg or >79.12 kg); and lab session (morning, afternoon, or evening). The cutoffs for weight and age were determined based on the population median estimates. Nationally representative means and standard deviations (including weighting factors) of lognormal distributions for each combination of these four variables were estimated using the VARGEN procedure in SAS-callable SUDAAN® (RTI International, RTP, NC; SAS version 9.3, SUDAAN version 11.0.0). SUDAAN's VARGEN procedure allows the standard deviation of a distribution to be estimated using Taylor Series Expansion while incorporating sample weights and study design information. See Table S2 for the estimated distribution statistics.

2.2 Generating distributions of spot perchlorate concentrations

The original BE approach uses fixed values to simulate a steady state BE, consistent with an exposure guidance level. If the guidance level is a POD or an adjusted POD (for interspecies differences), then the predicted value is called the "BE_{POD}". As shown in Figure 1, this estimate

is subsequently divided by an uncertainty factor (generally 10× to account for intraspecies differences) to get one final BE value. In contrast, our stochastic BE approach uses a Monte Carlo technique, allowing for variation in exposure scenarios, PK, and urine output/creatinine excretion (for urinary biomarkers only) to generate a distribution of BE_{POD}.

As part of this case study, the stochastic procedures described above (sections 2.1.2 through 2.1.4) were combined through a series of simulations in order to evaluate the greatest sources of variation in predicted BE_{POD} distributions. All calculations were carried out using MATLAB® version R2013b 8.2.0.701 (MathWorks, Natick, MA, Windows platform). Simulated individuals were based on 1617 adults (20+ years old) whose urinary perchlorate levels were measured during the 2001-2002 NHANES. These individuals were chosen as the basis for our simulations since several published studies have analyzed the same dataset (Blount et al. 2007, Lorber 2009, Yang et al. 2012).

Figure 1 shows the process for generating a BE_{POD} distribution. First, "exposures" at the POD were given to simulated individuals as described in section 2.1.2 (i.e., various meal events, meal times, and dose fractions). Next, "exposures" were used as inputs into the PBPK model, which was run to pseudo-steady state, with Monte Carlo sampling of sensitive parameters (as described in section 2.1.3). "Sampling times" were chosen based on each NHANES individual's lab session, with a constraint that the sampling time had to be at least 30 minutes after the most recent meal. (A figure illustrating the interaction between lab session [i.e., spot sampling time] and dose frequency/timing can be found in the supplementary material, Figure S3.) Next, the mass flow rate (μg/hr) into the urinary compartment at the selected sampling time was divided by a urine output (L/hr) and matching creatinine excretion rate (mg/hr) sampled from the stratified distributions described above to give the instantaneous urinary concentration (μg/L)

and creatinine-adjusted concentration (μg/mg), respectively. The urine output and creatinine excretion values came from stratified distributions, as described in section 2.1.4. To identify the greatest sources of variability to the BE_{POD} estimates, twelve distributions of BE_{POD} were generated by setting selected parameters to their "average" values and incorporating probabilistic sampling of the remaining parameter values (Table 1). In successive runs, the contribution of variability in exposure, PK, and urine output/creatinine excretion was evaluated singly, and then all together.

Cases 1-6 were one mpd simulations with an intake dose of 7 μg/kg/day at noon, 7 am, or a randomized meal time based on the ATUS data. Cases 7-12 were three mpd, each with a fraction of the total daily intake dose of 7 μg/kg/day, with the meal times either fixed (at 7 am, noon, 5 pm) or randomized based on the ATUS data. Fractions of the daily dose per meal were either fixed at one third for each meal (cases 7, 9, 10, and 11) or allowed to vary between 10% and 80% per meal (cases 8 and 12). Randomization of sensitive PBPK parameters was performed for cases 4, 6, 10, and 12. Randomization of urine output/creatinine excretion was performed for cases 5, 6, 11, and 12. Case 12 is assumed to represent the most realistic scenario in which individuals in a population eat three meals per day at different times, have different exposures in each meal (fractions of total daily dose, in this case), have different physiology and PK, and produce urine/creatinine at different rates (Table 1).

2.3 Statistical Analysis of BE Distributions

Distributions generated using the stochastic BE approach can help identify the greatest sources of variability in a biomarker distribution, and can be used to statistically evaluate biomarker data, including distribution tails, from observational human studies. For this case

study, simulated distributions of BE_{POD} were compared to perchlorate measurements from the 2001-2002 NHANES using standard scores (*z*-scores).

First, each NHANES urinary perchlorate measurement (concentration and creatinine-adjusted concentration) was log-transformed and then standardized to the mean and standard deviation, both known, of the log-transformed simulated BE_{POD} distribution. Under the assumption that these standardized NHANES values were from the BE_{POD} distribution, a one-sided *z*-test statistic was used to estimate their probability, γ. To identify only those who were most likely to have been exposed at or above the POD, individual perchlorate measurements from the 2001-2002 NHANES with $\gamma > 0.01$ were flagged for additional analysis. We chose this one percent probability as our lower bound cutpoint, in part, for robustness across repeated simulations of the BE_{POD} distribution (since the tails of the distribution are most subject to change subsequent to repeated probabilistic simulation). Other approaches for establishing a cutpoint, including non-parametric methods (e.g., flagging samples above the 1^{st} percentile of the BE_{POD} distribution), may also be considered when appropriate (e.g., a bimodal instead of a lognormal BE_{POD} distribution). Additional simulations were conducted for flagged measurements, using only matched demographic data (i.e., gender, age, body weight, and lab session), to generate targeted BE_{POD} distributions. This step essentially removes some inputs from probabilistic randomization during the Monte Carlo analysis. "Targeted γ" estimates were subsequently calculated by comparing individual perchlorate measurements with the corresponding targeted BE_{POD} distribution using the methods detailed above.

3. RESULTS

3.1 Population-Level Analysis of BE Distributions

Twelve simulated BE_{POD} distributions based on urinary perchlorate concentration are shown in Figure 2A. Medians of predicted distributions were fairly similar, ranging from 389 to 730 μg/L; these estimates are roughly two orders of magnitude higher than the median of the selected NHANES 2001-2002 sub-population distribution (3.8 μg/L). With the exception of case 2, medians based on the one mpd simulations (389 – 567 μg/L) were slightly lower than those based on the three mpd simulations (455 – 574 μg/L). Cases 7-10 produced the narrowest distributions, with ratios of the 95th percentile to the 5th percentile (95:5 ratio) ranging from 2.6 to 3.3; these three mpd cases varied one of three factors (i.e., dose fraction, dose timing, or sensitive PBPK parameters), or varied no factors. Cases 5, 6, 11, and 12 produced the widest distributions, with 95:5 ratio ranging from 18 to 48; these one or three mpd cases varied urine output, or varied all factors. The spread of distributions from cases 11 (95:5 ratio = 18) and 12 (95:5 ratio = 23) are most comparable to that of the measured 2001-2002 NHANES data, where the 95:5 ratio is 18.

Twelve simulated BE_{POD} distributions based on creatinine-adjusted perchlorate concentration are shown in Figure 2B. Trends here are very similar to those in Figure 2A. Specifically, medians were slightly (except in case 2) lower in the one mpd simulations (0.42 to 0.55 μg/mg) than in the three mpd simulations (0.52 to 0.56 μg/mg). The median creatinine-adjusted perchlorate concentration from the 2001-2002 NHANES is 0.0035 μg/mg, which is approximately 150-times lower than median estimates from the three mpd simulations. Again, the narrowest distributions were observed for cases 7-10 (95:5 ratios: 2.6 to 3.3). The widest distributions were observed in cases 5, 6, 11, and 12. Estimates of 95:5 ratios for these simulated

distributions ranged from 12 to 30, which is comparable to the estimate for the 2001-2002 NHANES data (95:5 ratio = 12).

Comparisons of case 12 simulated values and 2001-2002 NHANES data are shown in Figures 3A (for urinary perchlorate concentrations) and 3B (for creatinine-adjusted concentrations). Estimated geometric standard deviations (GSD) were comparable across NHANES and simulated distributions for both urine concentrations (NHANES GSD = 0.386 μg/L; simulated GSD = 0.412 μg/L) and creatinine-adjusted concentrations (NHANES GSD = 0.346 μg/mg; simulated GSD = 0.355 μg/mg). Estimated GM levels of the simulated BE distributions (479 μg/L and 0.523 μg/mg) were ~135× larger than those based on the NHANES data (3.64 μg/L and 0.0037 μg/mg).

3.2 Individual Subject-Level Analysis of BE Distributions

There is little overlap between the lower tails of the simulated BE_{POD} distributions (case 12) and the upper tails of the NHANES histograms (Figures 3A and 3B). A γ value was estimated for the individuals in the upper tails of the NHANES dataset. Six NHANES individuals were flagged with estimates of γ that were larger than 0.01. The two individuals with the highest urinary perchlorate concentration (100 μg/L) had γ estimates approximately equal to 0.05 (Table 2). That is, if these two individuals had been consistently and repeatedly exposed to perchlorate at the POD, there was about a 95% chance that the measured biomarker concentrations for these individuals would have been larger than 100 μg/L. For the creatinine-adjusted values, only two subjects had γ estimates greater than 0.01; these subjects also had γ estimates greater than 0.01 based on perchlorate concentration. As such, no new subjects were flagged based on their creatinine-adjusted concentrations. The γ estimates for these two subjects

increased when moving from concentration to creatinine-adjusted values. Since the observed creatinine concentrations for their samples were within an acceptable range of 30 – 300 mg/dL (WHO 1996), their high biomarker measurements were likely not due to dehydration or impaired kidney function. The γ estimate for highest ranked individual based on their creatinine-adjusted level (0.25 μg/mg) was 0.18, suggesting an 82% chance of observing a creatinine-adjusted concentration at or above 0.25 μg/mg given repeated daily dietary exposure at the POD.

The γ estimates for flagged individuals were recalculated using targeted BE_{POD} distributions. For these targeted distributions, some model inputs (such as body weight, age, and gender) were set equal to the values for a flagged individual, rather than being allowed to take on the full range of values found in the population. Little change (< 3×) was observed in γ estimates for four of the six individuals, whereas considerable decreases (more than an order of magnitude) in γ estimates were observed for the other two who had considerably greater body weights (Table 2). Since the simulated fixed dose (POD) was scaled by body weight, these two individuals received a larger total dose that shifted the BE distributions to the right, decreasing the extent of overlap and resulting in reduced estimates of γ.

Only two adults from the 2001-2002 NHANES were identified as having γ estimates greater than 0.01 considering population *and* targeted BE_{POD} distributions of both concentration *and* creatinine-adjusted values. Based on the targeted BE_{POD} distributions of creatinine-adjusted values, the largest γ estimates were 0.16 and 0.025; all other estimates were below 0.002. In other words, our simulation results suggest that for all but two of these NHANES subjects, there was at least a 99.8% chance of observing a higher biomarker concentration than what was actually measured if the daily dietary exposure had been at the POD.

4. DISCUSSION

The need to prioritize chemicals based on estimated risk is now at the forefront of public health and regulatory agendas (USEPA 2012, USEPA 2013b, USNRC 2007, USNRC 2012). Indeed, high throughput (HT) screening for exposure and/or toxicity is increasingly used to prioritize thousands of chemicals for further testing (Kavlock et al. 2012, Wambaugh et al. 2013, Wetmore et al. 2012). While the original BE approach is also intended as a screening tool, its purpose is distinct from that of HT research ─ it is meant to prioritize a smaller set of chemicals for which exposure guidance values and biomarker data exist. Based on the distance between biomarker measurements and corresponding BEs, chemicals are classified as low, medium, or high priority. Chemicals classified as medium or high priority may then require follow-up actions such as in-depth exposure assessment, risk assessment re-evaluation, or risk mitigation actions.

Deriving a BE requires an exposure guidance value and a mathematical description of the exposure-biomarker relationship. Interpreting biomarkers using a BE requires robust biomarker data (e.g., with most measurements above the detection limit), as well as methods for interpreting "spot" measurements against a chronic-exposure-based BE. (A notable exception is when the limit of detection is much lower than the BE and many biomarker levels are below the limit of detection. In this case, the large number of non-detects would not offset a conclusion of low risk.) The original BE approach relies on simplified exposure scenarios (e.g., continuous or bolus dose) and exposure-biomarker relationships (e.g., linear and deterministic) for BE derivation, and central tendency (e.g., GM) or upper percentile (e.g., 95^{th} percentile) estimates of measurement data for BE interpretation. Interpretations based on upper percentile estimates can be misleading for non-persistent chemicals due to rapid fluctuations in biomarker levels. This

issue makes cross-chemical evaluation and prioritization challenging. New methods are therefore needed to make informative comparisons between biomarker data and BEs (particularly when considering the distribution tails) with the goal of reducing uncertainty in prioritization decisions.

The stochastic BE approach can be adapted for analyzing biomarker data for virtually any compound. While Figure 1 illustrates the fundamental components of the approach, the specific structure of any component is subject to the availability of data. For instance, a simple one-compartment PK model could be used in lieu of a full PBPK model, and a multi-route exposure model could be used to replace the oral-only exposure model. The distinguishing feature of the stochastic BE approach is the consideration of variability in model parameters and predictions. As long as variability can be adequately addressed in any of the components, the approach can be considered a part of the stochastic BE approach.

There are three possible scenarios when prioritizing chemicals using the original BE approach. These scenarios are illustrated here using two HQ estimations: one based on geometric mean (HQ_{GM}) and the other based on a 95^{th} percentile estimate ($HQ_{0.95}$). If low values (<< 1) are estimated for both HQ_{GM} and $HQ_{0.95}$, then a chemical should be considered low priority and probably low risk. If high values (near or above unity) are estimated for both HQ_{GM} and $HQ_{0.95}$, then a chemical should be considered high priority and potentially high risk. In the third scenario, the estimate for HQ_{GM} is low (<1), but the estimate of $HQ_{0.95}$ is high (near or above unity). When multiple chemicals are observed in this category (as they were in Aylward et al. 2013), decisions must be made as to which chemicals are higher priority. To aid decision-making, the current study presents a stochastic BE approach that has the capability to evaluate individual biomarker measurements by comparing them to a stimulated distribution of BEs.

For this case study, probabilistic exposure, dosimetry, and urine output/creatinine excretion models were first used to generate distributions of BEs for perchlorate based on the POD of 7 μg/kg/day. Simulated BE distributions were then carefully examined to identify major contributors to BE magnitude and variability. Overall, urine output/creatinine excretion and frequency of exposure (i.e., number of meals per day) were observed to be the largest contributors to BE variability (see Figure 2). One mpd simulations (cases 1-6 in Table 1) produced wider BE distributions than did three mpd simulations (cases 7-12). For three mpd simulations, the fraction of the daily dose in each meal (case 8), specific timing of each meal (case 9), and PBPK parameters (case 10) all had relatively little impact on BE variability. The magnitude of BEs, as reflected by median estimates, was stable across different scenarios. However, three mpd simulations tended to produce higher median BE levels than one mpd simulations. Taken together, these results have implications for both the original and stochastic BE approaches. The original approach approximates continuous exposure, and our results suggest that this strategy could overestimate a BE, and underestimate a HQ, if intermittent exposures are expected for a given chemical. With respect to the stochastic BE approach, all future studies evaluating urinary biomarkers should take into account variability in urine output and/or creatinine excretion. Assuming constant rates for these values would severely underestimate the spread of the urinary biomarker distribution.

After simulating and evaluating BE distributions, a multi-step analysis was performed to interpret urinary perchlorate concentrations and creatinine-adjusted concentrations measured in the 2001-2002 NHANES. We compared the biomarker measurements from NHANES adults with the BE_{POD} distribution based on case 12, which best reflects reality (Figure 3). This case assumes that dietary perchlorate exposure occurs at meals, three times per day, with variations

across individuals in meal timing, dose fractions per meal, PBPK parameter values, and urine output/creatinine excretion values. The following discussion highlights key results from our analysis, as well as important distinctions between our results and those that would be expected from the original BE approach.

If we were to calculate HQs using the protocol of the original BE approach, a final BE for perchlorate could be calculated by dividing the GM of the simulated BE_{POD} distribution (479 μg/L or 0.523 μg/mg) by an uncertainty factor of 10. Then, this final BE could be used to estimate HQ_{GM} and $HQ_{0.95}$ using the GM and the 95th percentile of the NHANES biomarker measurements. Based on this approach, HQ_{GM} and $HQ_{0.95}$ are estimated to be 0.076 and 0.31, respectively, for urinary perchlorate concentrations, and 0.071 and 0.26, respectively, for creatinine-adjusted concentrations. Interestingly, these results suggest that perchlorate fits into the third scenario described above, where $HQ_{GM} < 1$ and $HQ_{0.95}$ is near unity. These HQ estimates underscore the need for an approach that could further prioritize perchlorate in relation to other chemicals with similar HQ estimates [e.g., di(2-ethylhexyl)phthalate, 1,4-dichlorobenzene, and benzene in nonsmokers (Aylward et al. 2013)].

There are several reasons why the original BE approach is better suited for comparing exposure *across* chemicals rather than evaluating the exposures of individuals to a single chemical. First, the original BE approach is meant for examining biomarker data from a population, and not from individuals. As discussed earlier, it can be challenging to interpret HQ estimates at the tails of measurements distributions, particularly when the biomarker of interest is short-lived. Second, the original BE approach indirectly compares biomarker data to a guidance value, rather than estimating exposures that are consistent with biomarker data. It may be

difficult to evaluate results based only on HQs for possible follow-up action(s). Setting acceptable cutoff values for HQs at the 50^{th} or 95^{th} percentile is not straightforward.

Several "exposure reconstruction" methodologies have been utilized to estimate exposure levels that are consistent with biomarker data. For example, Blount et al., Huber et al., and Mendez et al. have each estimated perchlorate exposure levels using the 2001-2002 NHANES biomarker data (Blount et al. 2007, Huber et al. 2011, Mendez et al. 2010). These studies benefit from framing results in terms of estimated exposure levels (rather than HQ), but are not without limitations. For example, in these studies each biomarker measurement was the basis for a "reconstructed" exposure value. Specifically, each spot measurement was assumed to be related to an individual's exposure by a single constant of proportionality. This approach contrasts with others that have been developed for exposure reconstruction, for instance the Exposure Conversion Factor approach where multiple constants of proportionality are estimated, which are then used to translate a single biomarker measurement into a distribution of exposures that are consistent with that measurement (Tan et al. 2006). Since spot biomarker concentrations are sometimes poor surrogates of longer-term averages (Pleil and Sobus 2013, Rappaport and Kupper 2008) these methods should only be considered appropriate at the population level when dealing with short-lived biomarkers. Thus, a need still exists for biomarker-based exposure assessment approaches at the individual level.

The stochastic BE approach does not attempt to reconstruct individual exposures from biomarker data, but instead statistically evaluates individual biomarker measurements with respect to exposure at a guidance value. Thus, in principal, the stochastic BE approach is very similar to the original BE approach. However, the stochastic BE approach goes beyond HQ

estimation to provide meaningful, quantitative insights into single chemical exposures; these insights are expressed probabilistically for each individual with a biomarker measurement.

Consider that a BE_{POD} distribution is intended to reflect the *possible* biomarker concentrations in a hypothetical population where all individuals are exposed equally, and repeatedly, at a POD. The stochastic BE approach is used to first generate the BE_{POD} distribution, and then to interpret individual biomarker measurements with respect to that distribution. Our results for perchlorate suggest at least a 99.8% chance, for all but two individuals in the 2001-2002 NHANES sub-population, of observing a higher biomarker concentration than what was actually measured, assuming daily exposure at the POD through the diet. Even if the most extreme individual (i.e., the one with the largest measured perchlorate concentration) had been exposed repeatedly at the POD, there was still an 84% chance of observing a higher biomarker concentration than what was actually measured. These probabilistic results help place the NHANES biomarker measurements into a quantitative exposure context, and suggest that perchlorate exposures among the 2001-2002 NHANES adults were likely well below the POD.

Estimates of γ were calculated for NHANES individuals using population and targeted BE_{POD} distributions, and both concentration and creatinine-adjusted values. The purpose of this iterative process was to ensure that elevated γ estimates were not artifacts of compromised biological samples (as evaluated by creatinine concentrations) or model restrictions. While our case study example uses urinary perchlorate measurements from the NHANES, the stochastic BE approach can certainty be applied to evaluate chemical biomarker data from other studies (e.g., targeted epidemiological studies). Here, the NHANES biometric data may still be useful for constructing population-level BE distributions based on certain variables (e.g., body weight). However, a lack of matched biomarker and biometric data may ultimately prevent the

construction of targeted BE distributions. Thus, investigators applying the stochastic BE approach should evaluate on a case-by-case basis whether targeted BE distributions can be calculated (leading to targeted γ estimates), or if interpretations must be based on more global BE distributions.

We note that our estimated BE_{POD} distributions may have been tighter, and our estimations of γ lower, if urine output and creatinine excretion values had been available for the 2001-2002 NHANES subjects (these values were sampled from stratified distributions of the 2009-2010 NHANES dataset – a dataset in which the urinary perchlorate levels are not yet available). Thus, future analyses will benefit from samples with matched chemical concentration, urine output, and creatinine concentration values. Ultimately, refined γ estimates for perchlorate should be compared to γ estimates for other chemicals where existing $HQ_{0.95}$ levels are near unity. This type of multi-chemical evaluation would support refined prioritizations for specific chemicals, and perhaps preliminary decisions regarding follow-up research/actions.

While the stochastic BE approach is useful for evaluating biomarker measurements in an exposure context, it is not intended to provide direct risk estimates for individuals. Rather, it is limited to identifying individuals whose biomarker concentrations have exceeded some evaluation criterion (e.g., $\gamma \geq 0.01$ in our example). Examination using quantitative criteria should be considered an important step in exposure evaluation, but not a final step in risk assessment, for several reasons:

(1) NHANES biomarkers are single spot measurements, and a spot measurement for an individual can fall much higher or lower than the average biomarker concentration for

that individual. Many guidelines are predicated on chronic, rather than acute exposures, complicating the analysis of spot measurements in relation to risk.

(2) In our example, the distribution of BE_{POD} from case 12 was selected since it was considered the most realistic. If a different case had been used with a much narrower distribution (e.g., case 3), then it is possible that no individual in the NHANES sub-population would have exceeded the evaluation criterion. If the same analysis had been performed using different model inputs or assumptions, it follows that different people could have been flagged as exceeding the evaluation criterion.

(3) In our example, six individuals had $\gamma \geq 0.01$ when using concentrations on a volume-basis; and two had $\gamma \geq 0.01$ when using creatinine-adjusted concentrations. While both are "urinary biomarker measurements," different ways of presenting the data result in different individuals being flagged.

(4) The number of individuals exceeding an evaluation criterion depends on the criterion. If the cutoff has been changed to $\gamma \geq 0.001$, than 29 individuals would have exceeded this new criterion (using concentrations on a volume-basis).

Item 1 is inherent in any effort to use spot biomarker data for understanding health risk, regardless of method, while items 2-4 are specific to our method. The stochastic BE approach has the advantage, being a computational technique, that it can be quickly and easily repeated as additional information becomes available and any underlying assumptions are reexamined or revised.

In summary, the γ estimate for each individual, as well as the number of individuals meeting an evaluation criterion, depend upon: a) the assumptions underlying the models, b) the parameters that are considered for BE derivation, c) the population(s) used for model calibration,

and d) the units of the biomarker output. Thus, the generation and evaluation of multiple distributions (given different assumptions and scenarios) is recommended prior to calculating final γ levels. The goal is to maximize confidence in both the central tendency and spread of the simulated BE distribution so that γ estimates are meaningful and facilitate comparisons across chemicals.

5.0 CONCLUSION

The stochastic BE approach was conceived as a "next step" in using biomarker data for chemical prioritization. Its primary value is in providing quantitative metrics, in addition to HQ estimates, to compare across chemicals to identify those most in need of additional research to characterize exposure, health and environmental effects, and/or overall risk. A secondary benefit of the approach is its ability to give statistical insight into single chemical exposures with respect to exposure at guidance values. Whenever possible, this approach should be used to: (1) explore exposure-biomarker relationships and their impact on BE estimates; (2) statistically evaluate biomarker measurements with respect to exposure guidance values; and (3) further examine chemicals in need of follow-up investigation based on existing HQ estimates.

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FIGURE CAPTIONS

Figure 1. Flowchart of the original BE approach (solid lines) and stochastic BE approach (dotted lines). Rounded rectangles denote the beginning and end of the approaches, parallelograms denote inputs, rectangles denote processes or models, circles denote connection points, and diamonds denote a branch (either/or).

Figure 2. Case Results: Distribution of BEs for (A) urinary perchlorate concentration (μg/L), and (B) creatinine-adjusted perchlorate concentration (μg/mg). Whiskers extend to the 5^{th} and 95^{th} percentiles, red bullets indicate the 1^{st} and 99^{th} percentiles, and red plus signs indicate the minimum and maximum values. See Table 1 for a summary of the experimental setup of each case.

Figure 3. Overlap between NHANES 2001-2002 urinary perchlorate biomarker data (sub-population) and the distributions of BE_{POD} (case 12). (A) Urinary concentration (μg/L). (B) Creatinine-adjusted urinary concentration (μg/mg)

Figure 1

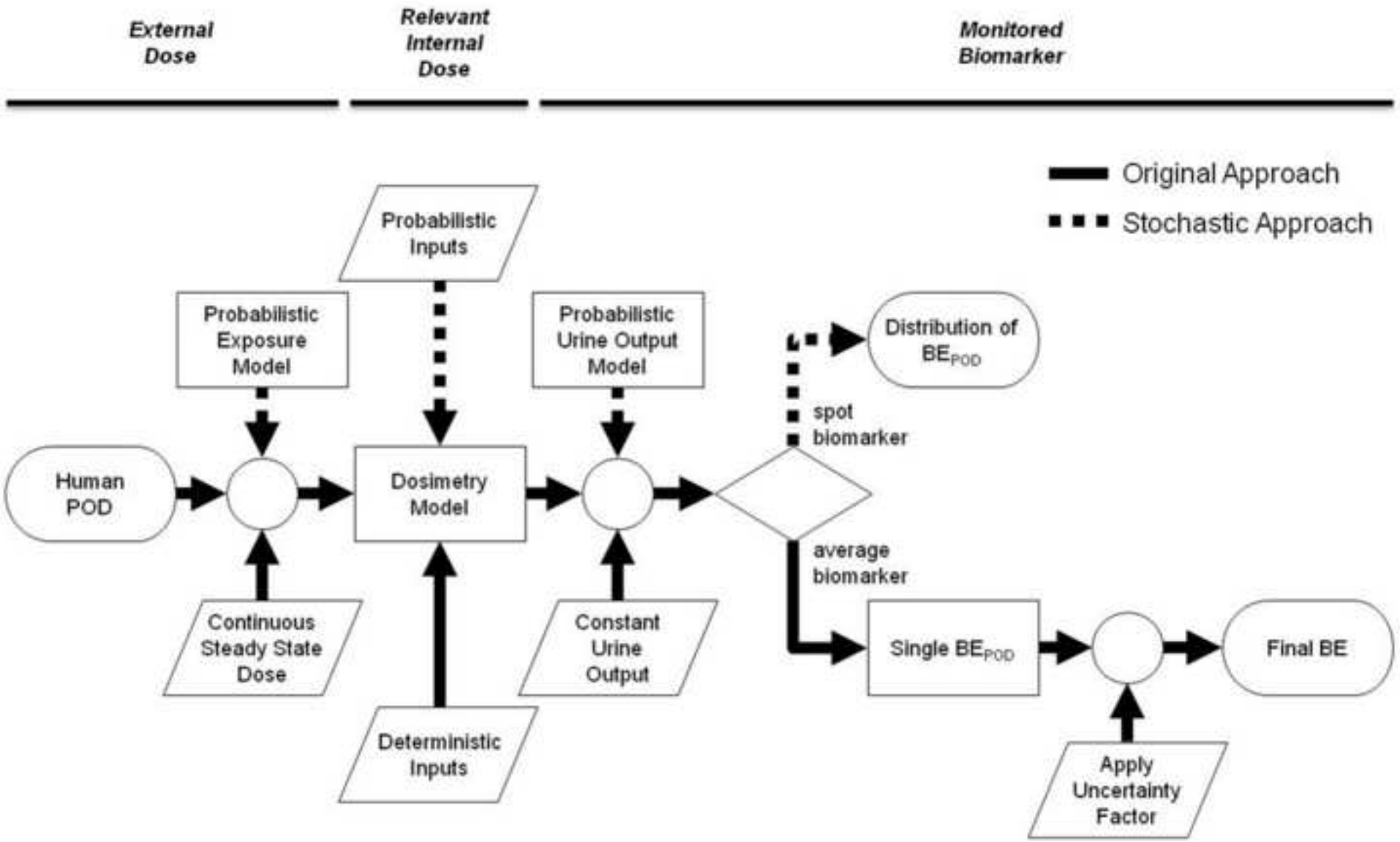
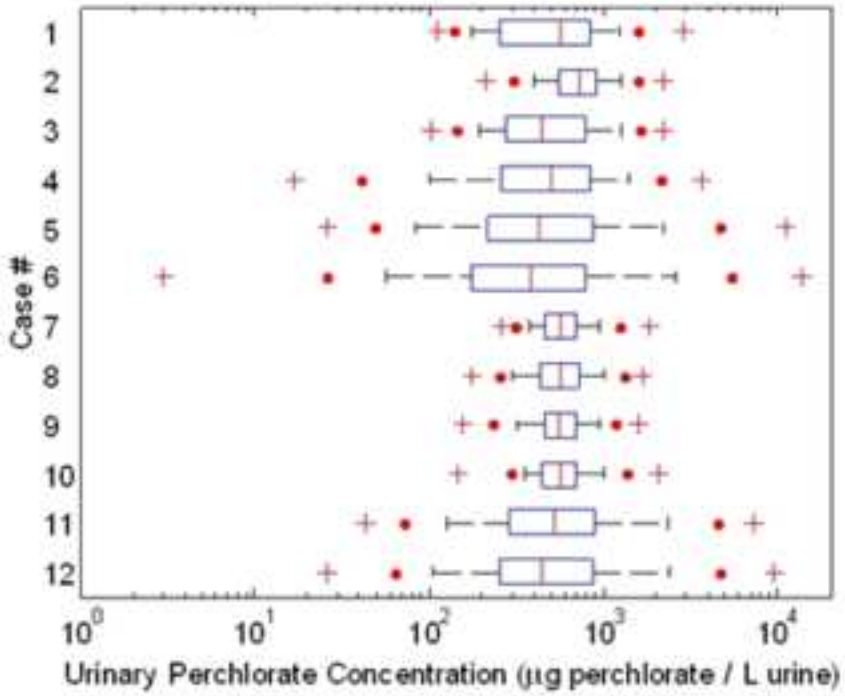
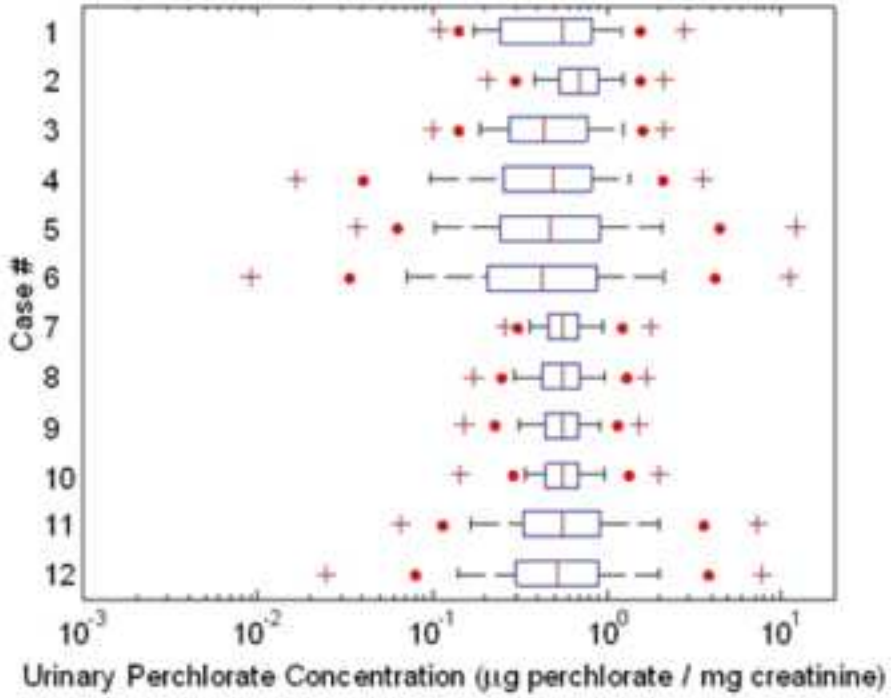

External Dose
Relevant Internal Dose
Monitored Biomarker
Original Approach
Stochastic Approach
Probabilistic Inputs
Probabilistic Exposure Model
Probabilistic Urine Output Model
Distribution of BE_{POD}
spot biomarker
Human POD
Dosimetry Model
average biomarker
Continuous Steady State Dose
Constant Urine Output
Single BE_{POD}
Final BE
Deterministic Inputs
Apply Uncertainty Factor

Figure 2

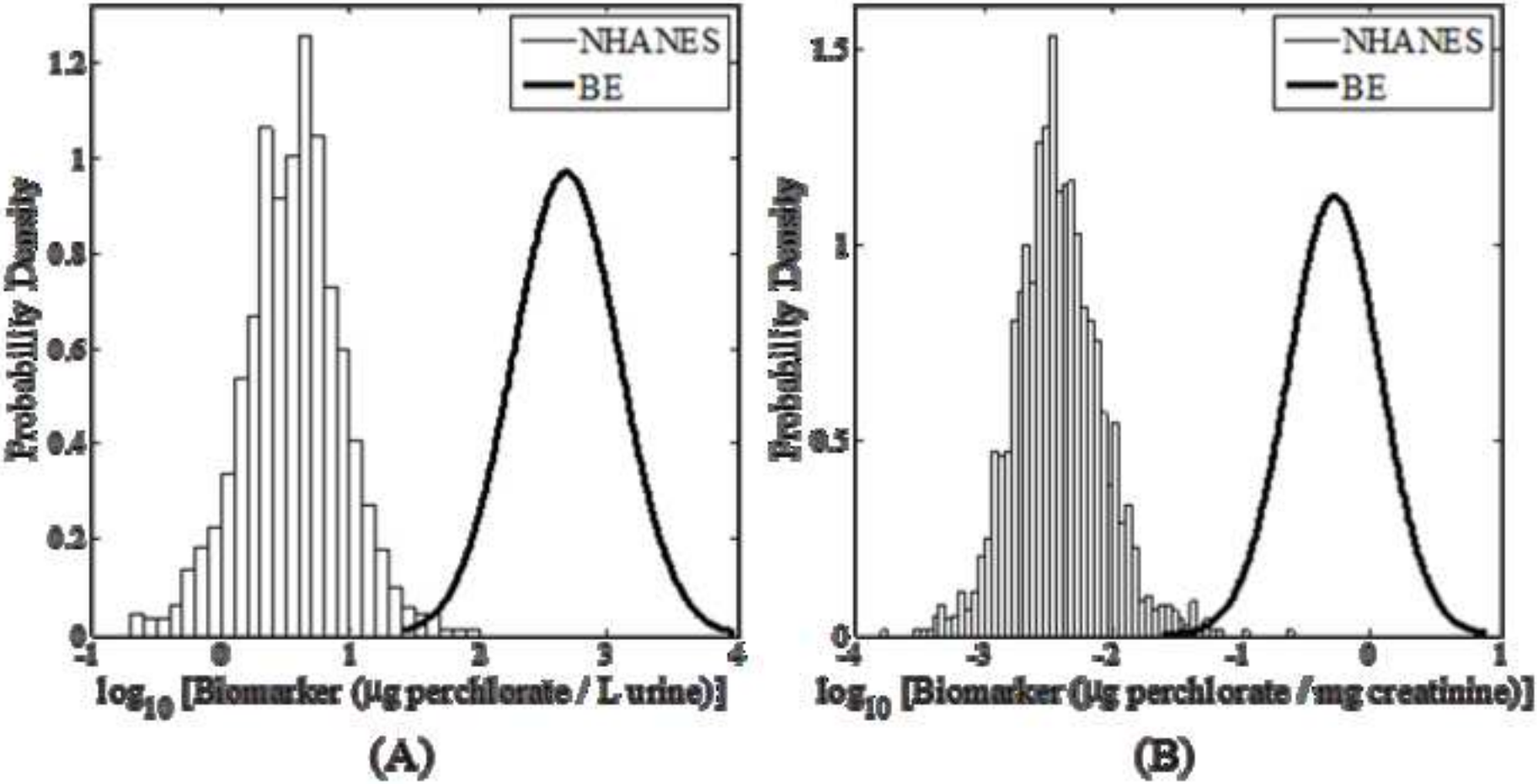

Case #
1
2
3
4
5
6
7
8
9
10
11
12
10^0
10^1
10^2
10^3
10^4
Urinary Perchlorate Concentration (µg perchlorate / L urine)

(A)


Case #
1
2
3
4
5
6
7
8
9
10
11
12
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
Urinary Perchlorate Concentration (µg perchlorate / mg creatinine)

(B)

Figure 3


NHANES
BE
Probability Density
log10 [Biomarker (µg perchlorate / L urine)]
(A)
NHANES
BE
Probability Density
log10 [Biomarker (µg perchlorate / mg creatinine)]
(B)

TABLES

Table 1. Summary of the differences among the twelve cases.

Case	mpd	Meal Time	PBPK	UO	Case	mpd	Variable Dose	Variable Time	PBPK	UO
1	1	12 PM	-	-	7	3	-	-	-	-
2	1	7 AM	-	-	8	3	x	-	-	-
3	1	random	-	-	9	3	-	x	-	-
4	1	12 PM	x	-	10	3	-	-	x	-
5	1	12 PM	-	x	11	3	-	-	-	x
6	1	random	x	x	12	3	x	x	x	x

Table 2. Results for NHANES 2001-2002 individuals who met the selection criterion (Population $\gamma \geq 0.01$).

SeqN	Gender	Age (yr)	Weight (kg)	Lab Session	Creatinine Conc. (mg/dL)	Biomarker Conc. (μg/L)	Population γ	Targeted γ	Creatinine-Adj. Conc. (μg/mg)	Population γ	Targeted γ
16435	Female	44	57.1	Afternoon	40	100	0.04929	0.05198	0.2500	0.18308	0.16373
13589	Male	29	162	Afternoon	149	100	0.04929	0.00667	0.0671	0.00599	0.00054
20821	Male	56	45.3	Afternoon	132	71	0.02205	0.05102	0.0538	0.00269	0.00673
10388	Female	59	129.5	Morning	261	71	0.02205	0.00235	0.0272	0.00015	9.09E-07
18071	Female	83	57.4	Morning	151	62	0.01554	0.03355	0.0411	0.00092	0.00163
17886	Female	23	49.7	Evening	60	60	0.01424	0.03373	0.1000	0.02144	0.02520