Biomarkers in Computational Toxicology

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Summary

Biomarkers are a means to evaluate chemical exposure and/or the subsequent impacts on toxicity pathways that lead to adverse health outcomes. Computational toxicology can integrate biomarker data with knowledge of exposure, chemistry, biology, pharmacokinetics, toxicology, and epidemiology to inform the linkages among exposure, susceptibility, and human health. This chapter provides an overview of four computational modeling approaches and their applications for interpreting biomarker data. Exposure models integrate the microenvironmental concentrations with human activity data to estimate intake doses. Dosimetry models incorporate mechanistic biological information to link intake doses to biomarkers. Biologically plausible models describe normal and xenobiotic-perturbed behaviors that can be distinguished using biomarkers. Cheminformatics-based models provide rapid assessments to inform future biomarker studies. Together these modeling approaches allow for comprehensive investigations of biomarker data to between link exposures and disease.

Key Words

Biomarker, computational toxicology, exposure, dosimetry, physiologically based pharmacokinetic model, biologically plausible model, chemical landscape

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Introduction

The term 'biomarker' encompasses many different, but related, definitions. This chapter applies the generally accepted categories of biomarkers from environmental sciences: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (IUPAC 2004, WHO/IPC 1993). This categorization directly relates biomarkers to a major goal of computational toxicology, i.e., linking exposure to health effects with the consideration of susceptibility to evaluate the health implications of exposures to environmental chemicals.

"Biomarkers of exposure" infer exposures to exogenous chemicals and are measured in accessible biological media, such as urine, blood, saliva, hair, or milk. A biomarker of exposure may be the chemical itself, its metabolite, or an endogenous species that changes predictably in response to exposure[1]. These biomarkers provide qualitative, and sometimes quantitative, information on changes in exposures over time or variability between different populations. When combined with appropriate computational models and exposure pathway information, it is sometimes possible to estimate the exposure concentrations that could have produced the measured biomarker concentrations, i.e., dose reconstruction. "Biomarkers of effect" are markers of biochemical or physiological changes that are indicative of biological responses to a chemical exposure. Some biomarkers of effect are known to be directly associated with specific adverse outcomes (e.g., cholinergic poisoning [Kim et al., 2010; Marsilach et al., 2011]), while others appear to be empirically associated with particular systemic effects (e.g., oxidative stress [Peluso et al., 2013; Zhang et al., 2013]). "Biomarkers of

[1] http://www.epa.gov/pesticides/science/biomarker.html

susceptibility" are markers of intrinsic susceptibility factors (e.g., genotype, gender, life stage) or extrinsic vulnerability factors (e.g., economic niche, individual choices, nutrition) that make some individuals more sensitive to chemical exposures. Both biomarkers of effect and susceptibility may be directly involved in the mode of action, which describes the sequences of key events that link tissue dose with an apical effect. These general distinctions are not always exclusive, and some biomarkers may fall into two or even all three categories. Others have proposed different systems of categorization (Tan et al., 2012); however, these three common categories are intuitive and will be used throughout this chapter.

Within the environmental sciences, dividing lines have existed between biomarker research in the context of exposure and effects identification needed for risk assessment. Exposure scientists generally utilize biomarkers of exposure to complement or substitute other exposure measurements; to estimate inter- and intra-individual variabilities in exposures; and to compare different groups of individuals. On the other hand, toxicologists generally utilize biomarkers of effect to investigate chemical/drug toxicity, preclinical effects, or changes in organ function. Whereas biomarker measurements can provide robust, independent tools to assess exposures or health risks, there is also a strong need and opportunity for trans-disciplinary, systems scientific research to integrate various biomarker data with other knowledge to better understand the effects of exposure (an external factor) and susceptibility (an internal factor) on human health. In particular, computational toxicology can systematically integrate knowledge and data from exposure, chemistry, biology, pharmacokinetics, toxicology, epidemiology, and biomarker measurements.

Computational toxicology is dedicated to developing and refining powerful techniques and paradigms to incorporate disparate classes of relevant information, including myriad data, knowledge-bases, statistical analysis, toxicogenomics, and predictive models. Its primary goal is to understand the public health impact of chemical exposures. This chapter presents an overview of several of these computational models and describes their application to interpreting or integrating biomarker data. Four types of models will be discussed: exposure models; dosimetry models; biologically plausible models; and cheminformatics models.

Exposure models integrate chemical concentrations in microenvironments with the amount of time an individual spends in these microenvironments to estimate the magnitude, frequency, and duration of contact with chemicals. Microenvironments are defined based on practical considerations, and may be as specific as the bathroom of a particular unit in an apartment complex or as general as all the single-family homes in a State. The simplifying assumption here is that exposures to the chemical of interest can reasonably be assumed to be uniform throughout the microenvironment.

The predicted exposure concentrations are sometimes used as surrogates for actual intake concentrations. Dosimetry models describe the biochemical processes that translate an exposure/intake dose into a delivered dose at a site inside the body. As a more specific example, physiologically-based pharmacokinetic (PBPK) models are often used to incorporate mechanistic data and physicochemical data to predict chemical-specific absorption,

distribution, metabolism, and excretion (ADME) in the body. As PBPK models are used mainly to predict the time course of chemical and sometimes its metabolite concentrations throughout the body, they naturally encapsulate the relationship between dose and biomarkers of exposure. Other biologically plausible models have the potential to provide quantitative estimates of biomarker-based risk; this potential may require development of new biomarkers suggested by recent developments in systems biology and toxicity pathway analysis. One example is a model that describes the normal and chemical-perturbed behaviors of signaling pathways (Bhalla et al., 2002; Hoffman et al., 2005). These systems-level models provide the opportunity to integrate biomarker data and other databases to identify system behavior that is unlikely to be discovered without these models. Finally, cheminformatics-based models can be used to analyze the chemical space of biomarkers, indicating the chemical space that have proven most indicative of exposures, the small coverage space of current biomarker knowledge, and the common transformations between parent chemical and biomarker. Generally, computational models strongly support the analysis and interpretation of existing biomarker data, which leads to a better understanding of chemical exposures, disease states, and susceptibility in human populations.

Exposure Modeling

Biomarkers of exposure are not direct measures of exposure; nonetheless, there is vast and growing interest in utilizing exposure biomarker data for exposure and risk assessment, especially as the number of biomonitoring studies increases (e.g., National Health and Nutrition Examination Survey [NHANES]). Biomarker measurements may be used to estimate exposure

concentrations only when one can quantitatively define the intake dose-biomarker relationship (Vallero, 2010). Intake dose is difficult to quantify for individuals, especially when there are multiple sources of exposure (Sobus et al., 2010). Thus, predictions generated using an exposure model are often used as a surrogate for the intake dose (Williams et al., 2010), and sometimes are taken a step further and are used for absorbed dose as well (Tornero-Velez et al., 2012), which is traditionally calculated as a dose multiplies by bioavailability. Exposure models (e.g. E-FAST[2], RAIDAR[3], USEtox[4], ConsExpo[5]) take as inputs data like source of exposure (e.g., food, air, water, consumer products, home microenvironments), route of exposure (e.g., inhalation, dermal contact), and other factors (e.g., hand-to-mouth frequency, hand-wash frequency, shower length, water intake per day). Exposure models vary considerably in their complexity; some are deterministic point estimates, and some are probabilistic to account for intra- and inter-individual variability in exposure patterns.

In addition to physicochemical and biological data, social sciences also contribute important exposure information. In its simplest form, exposure is integration of contact with a substance over time. The time includes frequency, duration, and magnitude of that contact, which is largely a function of where a person is and what a person is doing (Vallero et al., 2010). As a result, human activities contribute significantly to variability in exposure, probably more so than environmental concentrations. While human behaviors may appear to be arbitrary or random, certain patterns of behavior are known to be representative of different groups. For

[2] http://www.epa.gov/oppt/exposure/pubs/efast.htm
[3] http://www.trentu.ca/academic/envmodel/models/RAIDAR100.html
[4] http://www.usetox.org/
[5] http://www.rivm.nl/en/Topics/Topics/C/ConsExpo

example hand-to-mouth behavior among children below the age of 5 is significantly different than among the rest of the population (EPA 1997). For those behaviors that are more general, data may exist in database such as the Exposure Factor Handbook (EPA 1997), or Continuing Survey of Food Intake by Individuals (CSFII), or may be collected from new observational studies. Besides human activities, chemical concentration in the microenvironment or local media (e.g., air, food) is another key input to an exposure model. These concentrations may be measured directly (e.g., duplicate diet study, air monitoring) or may be found in observational surveys, such as the Total Diet Study (TDS) conducted by the U.S. Food and Drug Administration (FDA). TDS measures chemical (e.g., pesticides, arsenic, lead, cadmium, mercury) residue concentrations and nutrient elements in foods. Exposure models take chemical concentrations and human time/location activities as inputs to characterize potential exposure pathways and to aggregate all exposure sources.

Outputs of exposure models are often time and concentrations of exposure, which can then serve as inputs to dosimetry models for predicting biomarker concentrations to compare with measured data. For example, Choudhury and colleagues (2001) linked the Cadmium Dietary Exposure Model with Kjellstrom and Nordlbery's biokinetic model to simulate dietary intake of cadmium and corresponding urinary cadmium concentrations. Carrington and colleagues (2002; 2004) developed computational models to link seafood consumption to methylmercury concentrations in blood and hair samples from women and children. McKone and colleagues (2007) used the CalTOX multimedia, multipathway, source-to-dose model to predict total intakes of organophosphorus (OP) pesticides and urinary dialkylphosphate (DAP) metabolites

for pregnant Latina women in the Salinas Valley region. Xue and colleagues (2010) combined the Stochastic Human Exposure and Dose Simulation Dietary (SHEDS-Dietary) model with the Modeling Environment for Total Risk with Physiologically Based Pharmacokinetic Modeling for Population (MENTOR-3P) system to simulate dietary intake of inorganic arsenic, as well as corresponding urinary arsenic concentrations. Xue and colleagues (2012) also used the SHEDS-Dietary and a simple pharmacokinetic model to simulate methylmercury concentrations in fish and in blood.

Other studies have used combined exposure-PBPK models to predict biomarker concentrations. Tornero-Velez et al. (2012) linked a human PBPK model for permethrin to the SHEDS-Multimedia model to estimate permethrin exposures in the U.S. population from residential, dietary, and combined residential-dietary exposure routes. The SHEDS-Multimedia model used dietary consumption data, permethrin residues in food, residential concentrations, and other exposure factors (e.g., surface-to-skin transfer efficiency, hand mouthing frequency, fraction of house treated with insecticides) to predict exposure concentrations/profiles for 8994 individuals who were 6 years of age and older. The output exposure concentrations-time profiles from SHEDS-Multimedia were then used as inputs for the human PBPK model to predict urinary metabolites of permethrin. These predicted metabolite concentrations were compared to observed biomarker data to evaluate the ability of the linked SHEDS-PBPK model for simulating urinary metabolite concentrations in a population. Similar studies were found on biomarkers for other chemicals, such as trichloroethylene (Liao et al., 2007), chlorpyrifos (Lu et

al., 2010), polychlorinated biphenyls (Redding et al., 2008), cadmium (Ruiz et al., 2010), and perchlorate (Yang et al., 2012).

Physiologically-Based Pharmacokinetic (PBPK) Modeling

Dosimetry models simulate the disposition of chemicals at internal sites of the body; they have the capability to link external exposure or applied doses to internal exposure biomarker concentrations. There are many different types of dosimetry models, such as respiratory tract dosimetry models that predict the respiratory tract dosimetry of inhaled gases and particulates, intestine dosimetry models that predict the absorption of contents into the intestinal wall, or PBPK models that predict chemical-specific ADME. This chapter outlines how dosimetry models, in particular PBPK models, can be used to interpret exposure biomarker data, such as those measured in the National Health and Nutrition Examination Survey (NHANES), in the context of exposure or health risk.

NHANES is an ongoing national survey conducted by the Centers for Disease Control and Prevention (CDC) to obtain nationally representative information on medical histories, food consumption, and health/nutritional status of the population in the U.S. In addition to these demographic and health-related data, in 1999, NHANES[6] started to include exposure biomarkers of environmental chemicals, such as pesticides, heavy metals, and volatile organic compounds. These biomarkers are measured as a snapshot of a subsample's internal (e.g., blood) or excreted (e.g., urine) concentration; they are designed to identify exposure status and

[6] http://www.cdc.gov/biomonitoring/

allow analysis of trends in a population. These data are not specifically intended for exposure or risk assessment. However, for many chemicals these exposure biomarkers are the only source of data for chemical exposure. Thus, there is much interest, including from regulatory agencies, in using these valuable human data to assess both exposure sources and potential health risks. As is commonly a challenge in "found" data, care must be taken in applying them in ways for which they were not originally intended.

Repurposing biomonitoring data for hazard identification and risk assessment is challenging for a number of reasons. A single spot biomarker measurement cannot be used to distinguish the relative contributions from different exposure sources/routes, e.g., food and water (oral); sprays and creams (dermal); smog and aerosols (inhalation). Also, the relationship between exposure concentrations and biomarker concentrations is often complex; it is a function of pharmacokinetics, exposure patterns, biomarker collection protocol (e.g., blood vs. urine; spot urine vs. first morning void), and many other factors. For example, the biomarker concentration may reflect recent exposures, exposures averaged over some period of time, or a situation that is more complicated than either of these two cases.

Two examples below illustrate this challenge. The first is a simple case with the following conditions:

1. For chemical X, the exposure sources are meat and vegetables and ingestion is the only route of exposure. All ingested dose of X is absorbed from the GI tract with linear kinetics.
2. The biomarker of X is the chemical itself, and it is only measured in urine.

3. The blood concentration can be used as a surrogate for the target tissue concentration, but it is not measured in this biomonitoring study.
4. The half life of chemical X in blood is about four hours.
5. An individual was exposed to chemical X at breakfast; lunch (6 h after breakfast); dinner (6 h after lunch); and snack (2 h after dinner). This individual had five urine voids within a 24 h period (at time 3, 7, 11, 16, 23 h after breakfast).

The time course of the amount of chemical X in blood and urine during the 24 h period after the breakfast (Figure 1) are simulated using a classical pharmacokinetic model. In this example, the time course is simulated for a single subject, and thus, inter-individual differences in pharmacokinetics and urine excretion can be ignored. Here, the surrogate of target tissue dose is chemical X in blood (not measured), while the only biomarker is chemical X in urine measured only at void time (i.e., no urinary catheterization). Because urinary biomarkers are measured and reported as concentrations not amount in the real world, PBPK models calculate urinary concentration from the amount of biomarker accumulated between the time of two voids. Thus, a urinary biomarker concentration does not correlate solely with the intake dose; it also correlates with the time between voids, urine volume, chemical resorption, and other factors. In this simple example, the ADME- and urine-related parameters are held constant to illustrate how an urinary biomarker measurement is dependent upon the interaction of the dosing time, sampling time (concurrent with each void event), and the elapsed time between the two. From the simulation, the amount of chemical X in the first three voids was about the same, but they were the result of different combinations of doses and time between doses and

sampling. The fourth void had the highest amount of chemical X, which appropriately reflected the comparatively higher dose at dinner. The last void also had more chemical X than the first three voids. This was a result of the high dose at dinner, the additional dose from the after-dinner snack, and the continuous accumulation of biomarker in the urine overnight. Note that the urinary biomarker measurements did not show a strong correlation with the amount of chemical X in blood, which is a surrogate for target tissue dose and potential for health risks. This is the rule, not the exception, for chemicals with short half lives.

Under real-world conditions, many toxicants of interest require metabolic activation. So, the second example is a case with the following conditions:

1. For chemical Y, the exposure sources are meat and vegetables and ingestion is the only route of exposure (i.e. dietary exposure). All ingested dose of Y is absorbed from the GI tract (assuming 1st order kinetics).
2. The metabolite of Y, M1, causes liver toxicity at high doses, with the risk of toxicity being proportional to the level of M1.
3. The biomarker of Y, M2, is a metabolite of M1. M2 is the only species measured in urine and the level of M2 is always proportional to that of M1.
4. The half lives of Y and M1 are approximately equal and on the order of a few hours.

The biomarker concentrations and potential health risks for two individuals, A and B, can be compared under the following four scenarios (Table 1):

1. The elapsed time between exposure and sample collection, urine volume, metabolic rates and other ADME parameters, and physiological characteristics of the individuals were identical between A and B. The only difference was that A ingested a larger dose than B did of chemical Y. In this case, A will have a higher biomarker concentration and a higher risk than B. Thus, biomarker M2 is a good marker for both exposure and health risk.

2. Intake dose, the elapsed time between exposure and sample collection, urine volume, metabolic rate for the conversion of M1 to M2, other ADME parameters, and physiological characteristics were identical between A and B. The only difference was that A metabolized Y to M1 much faster than did B. In this case, A will have a higher biomarker concentration and a higher risk than B. Thus, biomarker M2 is not a good marker for exposure (same intake dose, but different biomarker concentrations), but a good marker for health risk.

3. Intake dose, the elapsed time between exposure and sample collection, urine volume, metabolic rate for the conversion of Y to M1, other ADME parameters, and physiological characteristics were identical between A and B. The only difference was that A metabolized M1 to M2 much faster than did B. In this case, A will have a higher biomarker concentration but a lower risk than B. Thus, biomarker M2 is not a good marker for either exposure or health risk.

4. Intake dose, the elapsed time between exposure and sample collection, metabolic rates and other ADME parameters, and physiological characteristics were identical between A and

B. The only difference was that A drank more water and therefore produced a larger volume of more dilute urine than B. In this case, A will have a lower biomarker concentration (non-creatinine adjusted) but same risk as B. Thus, biomarker M2 is not a good marker for either exposure or health risk.

Indeed, none of these four scenarios are entirely representative of real-world conditions. It is impossible for two people to have the same exposure dose to a specific chemical at the same time, have the exact same physiological characteristics, ADME behavior, or have produced the same volume of urine at the same time for every void - all these factors vary between individuals (case 5). Thus, a single spot biomarker measurement cannot be used accurately to determine exposure sources, routes, magnitude, frequency, duration, etc. Linking biomarker data to potential health risks can be an even more challenging task. First, there are many chemicals for which a biomarker has been developed but toxicity has not been evaluated. Without toxicity information, it is impossible to establish an exposure-effect, let alone a biomarker-effect, relationship. Even for chemicals with established toxicity values, biomarkers of exposure may have little or no correlation with the concentration of the biologically-active form at the site of toxicity, and thus with the health effects of interest, as shown in the two examples above.

Toxicity values are frequently derived from animal dose-response studies, as human subject testing with non-therapeutic chemicals is often not possible. In animal studies, chemicals are administered using a specified route, and exposures are controlled with respect to dose,

frequency, and duration. In addition, most animal studies are conducted one chemical at a time (neglecting mixture effects) with laboratory strains of animals that minimize inter-individual differences; target tissue doses and responses can be measured in animals while alive or during necropsy. In contrast, humans are simultaneously exposed to multiple chemicals at a continuum of doses, by various routes, and at a range of frequencies, durations, and magnitudes. There is much greater variability in human physiology and time/location behaviors, which are difficult, if not impossible, to measure directly for each individual. Additionally, biomarkers are only measured in accessible biological media (e.g., blood, urine, hair); they do not usually reflect total body burden or target tissue dose. The disconnection between exposure and hazard information leads to data gaps and uncertainties in using exposure biomarker data for assessing exposures or human health risks.

The time course relationship between intake doses and biomarker concentrations is determined by the physicochemical properties of the substance being studied, such as solubility in blood and tissues or susceptibility to biotransformation, along with the biological characteristics of the test subject, such as tissue volumes, blood flows, or metabolic rates. Since PBPK models have the capability to integrate these chemical and biological properties, they can be used to simulate the time course of biomarker concentrations in the subject at various intake doses and from all sources and routes. And, most PBPK models were developed using data from animal toxicity studies, so they have the capability to predict target tissue doses or even the corresponding toxic responses if they are linked to a biologically-based

dose-response (BBDR) model. Thus, PBPK models may be used to place exposure biomarker data in a risk context using two main approaches:

(1) Forward dosimetry: estimating biomarker concentrations based on exposure concentrations that characterize the toxicity of a chemical.

(2) Reverse dosimetry: estimating exposure concentrations based on biomarker concentrations for comparison to an exposure guidance value.

Pharmacokinetic Modeling: Forward and Reverse Dosimetry

Exposure guidance values are usually set based on animal toxicity studies. For instance, a cohort of rats could be exposed to 0, 2, 4, or 8 mg/kg of a chemical every day for two years. If animals die early, their internal organs are examined for abnormalities. Target tissues are identified and additional studies are performed to determine the dose when the first effects appear. In humans, however, most target organs cannot be examined directly, so some appropriate biomarker is measured instead. A rule of thumb is the more accessible the better, hence hair and toenails are better than urine, which is better than blood, which is much better than a liver biopsy. Biomarkers present a challenge to interpretation: how does one compare an external exposure concentration to an internal biomarker measurement? A forward dosimetry approach that has become increasingly popular is the Biomonitoring Equivalent (BE) approach.

The BE approach uses pharmacokinetic models or linear equations to convert an existing exposure guidance value to a biomarker concentration, called the BE (Hays et al., 2007, 2009;

Becker et al., 2012; Boogaard et al., 2011). The BE is a function of 1) the chosen endpoint, 2) the model assumptions, and 3) the chosen biomarker. Given a chemical known to target the lung, the BE for lung cancer using a model calibrated on full-grown adults using the principal urinary metabolite is going to be different than the BE for lung fibrosis using a model calibrated on children for the parent chemical in plasma. In the absence of an exposure guidance value, a point of departure (e.g., no observed adverse effect levels [NOAELs]) is used in conjunction with adjustment factors (also called uncertainty factors) to derive a BE. Once a BE is calculated, it can be divided by measured biomarker data to calculate a margin of safety, which can be used to group chemicals into low, medium, or high priority for risk assessment follow-up. To date, BE values have been derived for approximately 100 chemicals of various half-lives based on different toxicity endpoints (Becker et al., 2012; Angerer et al., 2011).

Another approach for placing exposure biomarkers in a risk context, reverse dosimetry, is the process of converting biomarker measurements into exposure concentrations or intake doses. The converted exposure concentrations can then be compared to "safe or acceptable" exposure concentrations derived from animal toxicity studies. Reverse dosimetry uses the dose-biomarker relationship described by a model along with measured biomarker concentrations to determine the plausible exposure concentrations. Reverse dosimetry is not the same as running the same models used for forward dosimetry in reverse. When dealing with systems of nonlinear differential equations, like in PBPK models, this would not even be possible. Rather, the process "reverses" the outputs of forward dosimetry using statistical and other tools that can vary in their level of sophistication. Examples of different reverse

dosimetry approaches include optimization or trial-and-error (Mosquin et al., 2012; Roy et al., 1998), ratio calculations (Tan et al., 2006; Georgopoulos et al., 2009), and Bayesian methods (Allen et al., 2007; Georgopoulos et al., 2009; McNally et al., 2012; Sohn et al., 2004; Tan et al., 2007). The reliability of the exposure/dose estimates from reverse dosimetry approach depends on the accuracy of the dose-biomarker relationship described by the model, the information on exposure scenarios and their quality, and information on biomonitoring study design (e.g., time of sampling, first morning void vs. spot urine samples). At the population level, probabilistic reverse dosimetry incorporates variability and uncertainty in chemical pharmacokinetics and exposure patterns to infer exposure concentrations/doses from population-based biomarker measurements. In the next section, an example of probabilistic reverse dosimetry is given using a PBPK model for perchlorate.

Probabilistic Reverse Dosimetry

Reverse dosimetry involves two steps: (1) formulating a model that describes the dose-biomarker relationship as a function of time to use when performing forward dosimetry simulations; and (2) solving for the plausible doses that are consistent with observed biomarker concentrations. Probabilistic reverse dosimetry accounts for uncertainty and interindividual variability in exposure patterns and pharmacokinetics to estimate a distribution of exposure concentrations likely to have produced the observed biomarker concentrations in a population. Monte Carlo analysis is a common tool for generating the probabilistic distributions of the dose-biomarker relationship. In this example, probabilistic reverse dosimetry was conducted to estimate the distribution of average daily intake doses of

perchlorate based on urinary perchlorate concentrations measured for adults age 20 and older (1617 individuals) in the NHANES 2001-2002 survey. Perchlorate was selected because of the abundance of both exposure and biomarker data and the existence of a PBPK model (EPA 2009). In addition, its dose-biomarker relationship is rather simple: it can be assumed that (1) perchlorate is completely absorbed; (2) there is no metabolism; and (3) 100% of the dose is excreted in the urine, with urinary perchlorate being the biomarker.

In this example, the body weight of each individual was used to scale the tissue volumes, cardiac output, active transport parameters, partition/area cross-products, and urinary clearance rate in the PBPK model. A value of 70 kg was assigned to those with missing body weight data. Men were assigned an average body fat of 21.3%; and women were assigned an average body fat of 32.7%. A sensitivity analysis of model parameters was performed to identify those parameters with the greatest impact on the calculated urinary concentration. Estimates of uncertainty and variability were taken from the literature; in the absence of published values, a coefficient of variance of 50% was assumed. Next, each simulated subject was administered a single daily dose at noon for seven days, assuming the same daily dose each day. The simulation generated the time course of perchlorate urinary concentration for each individual; these were sampled during the 24 h window after the last dose using a uniform random distribution of sample times. The end result was a biomarker concentration for 1617 individuals at 100 different daily doses of perchlorate ranging from 1 ng/kg/day to 700 ng/kg/day (161,700 simulated spot biomarkers). These data were combined with the actual biomarker measurements published in NHANES 2001-2002 to give the most

likely distribution of real-life exposures using a web-based tool, Probabilistic Reverse dOsimetry Estimating Exposure Distribution (PROcEED)[7].

There are two probabilistic reverse dosimetry methods currently included in PROcEED: the Exposure Conversion Factor (ECF) and Discretized Bayesian Approach (DBA). ECF converts measured biomarker concentrations to distributions of exposure concentrations (or intake doses) by assuming that the dose-biomarker relationship is linear. In this method, the simulated population is exposed at a dose of 1 unit using forward dosimetry. The unit of the dose (e.g., 1 ng/kg/day) will determine the unit of the model predicted biomarker concentrations; this setting allows the flexibility to choose the most appropriate units. Then, PROcEED takes the reciprocals of predicted biomarker concentrations to generate a distribution of exposure conversion factors (ECFs). The ECFs are then multiplied by measured biomarker concentrations to obtain a distribution of estimated exposure concentrations or intake doses (Figure 3). In contrast, DBA is a more robust approach that uses the Bayesian inference. This method relies on several forward dosimetry simulations to capture any nonlinearity in the dose-biomarker relationship. It also has the potential to generate more accurate predictions if one has good knowledge on the priors (e.g., have some environmental measurements).

In our perchlorate example, both ECF and DBA predicted the most probable intake dose that this NHANES population received was about 50 mg/kg/day. For ECF, the geometric mean (GM) was 52.33 ng/kg/day, with a geometric standard deviation (GSD) of 2.89. DBA gave virtually

[7] http://www.epa.gov/heasd/products/proceed/proceed.html

identical results (GM: 51.60 ng/kg/day, GSD: 2.92). Other studies have also independently estimated the median intake doses using a variety of methods, such as multiplying the concentration of perchlorate in foods by their consumption rates. The average intake reported by these more traditional exposure studies ranged between 53 - 390 ng/kg/day (Blount et al., 2007; Murray et al., 2008; Lorber 2009; Sanchez et al., 2009; JECFA 2010; Mendez et al., 2010; Huber et al., 2011). Comparing PROcEED to these methods, the probabilistic approach has the advantage of giving the full distribution of possible doses, not just an estimate of the central tendency (Figure 4).

Biologically Plausible Modeling

Up to this point, the discussion has focused on linking biomarkers to exposures using computational models. As computational toxicology models rely more on high throughput screening (HTS) results (Kavlock, 2010; Rusyn, 2010), biomarkers of health effects and susceptibility will play a critical role in bridging those early molecular changes to organism and population level effects used for regulatory purposes. The integration of HTS and biomarker data to adverse health outcomes depends on the use of biologically plausible models that provide quantitative estimates of dose-based risk, predictive of potential impacts on human health. Building these computational models may require discovery of novel biomarkers suggested by new developments in systems biology and toxicity pathway analysis.

The number of chemicals being produced has overwhelmed the traditional mechanisms for toxicity testing. In 2007, the U.S. National Research Council (NRC, 2007; Andersen, 2010;

Krewski, 2011) recommended a transition to in vitro HTS assays with a capacity to evaluate hundreds of thousands of chemicals affordably. They defined the concept of toxicity pathways as "interconnected pathways composed of complex biochemical interactions of genes, proteins, and small molecules that maintain normal cellular function, control communication between cells, and allow cells to adapt to changes in their environment" that "when sufficiently perturbed, are expected to result in adverse health effects" (NRC, 2007). These upstream molecular and cellular effects can be monitored in vitro providing a high throughput method for chemical screening and prioritization. However, with additional mechanistic information to link these upstream events to the downstream adverse outcome, often referred to as the apical effect, the possibility exists for regulations based on the HTS data alone (Cote, 2012).

The adverse outcome pathway (AOP) was developed to provide a conceptual framework for assembling the mechanistic information needed to link the toxicity pathway perturbation to adverse outcomes of regulatory concern (Ankley et al., 2010). The AOP concept is very analogous to the mode of action (MOA) framework developed primarily for human health risk assessment (Boobis et al., 2008). The primary differences are the explicit inclusion of toxicity pathways and the extension of the AOP to include population level effects used in ecological risk assessments. An AOP consists of a molecular initiating event (i.e. perturbation of a toxicity pathway), followed by a series of intermediate events, and culminating in an adverse outcome at the organism or population level. Those intermediate events identified as necessary for the adverse outcome are considered key events as defined in the MOA framework. Quantitative

information regarding these key events is needed before toxicity pathway perturbations can be used to predict regulatory outcomes.

To provide this quantitative link between toxicity pathways and adverse outcomes, computational models will be iteratively refined as new data and new degrees of understanding of the relevant biological processes become available. Numerous efforts are underway to develop virtual tissues and even virtual organisms (Adra et al., 2010; Shah and Wambaugh, 2010), where multiple scales of biology – molecular, macromolecular, organelle, tissue – are described in a spatially and temporally realistic manner. Such models, with sufficient validation, will generate useful predictions of biological behaviors and toxic responses that today can only be examined in the wet laboratory using *in vitro* and *in vivo* methods. While important and useful, these models are, however, preliminary steps towards development of virtual tissues that can actually replace their living equivalents. In the meantime, the computational toxicology will continue to evolve and play an increasingly important role in toxicological research and human health risk assessment.

Changes in the concentration of some biomarkers may signal significant biological changes in both target and non-target tissues. Understanding these relationships will help support the development of computational models to predict the magnitude and duration of any health effects. Some biomarkers are capable of distinguishing between compensatory mechanisms that aim to restore homeostasis, and critical health effects with more serious and/or lasting consequences. Together with computational models, these biomarkers can be integral

components of toxicity pathway models. For example, signaling pathways, like the mitogen-activated protein kinase (MAPK) pathway, may be evaluated for potential biomarkers (Bhalla et al., 2002). Signaling pathways consist of one or more receptors at the cell surface that, when activated by an appropriate ligand, transmit signals to cytosolic effectors and also to the genome. The cytosolic effects are rapid, occurring within seconds or minutes of receptor activation, while the effects on gene expression take longer, with changes in the associated protein levels typically occurring after one or more hours. These pathways are characterized by complex feedback and feedforward mechanisms that can attenuate or amplify the signal and modulate the end result in a significant way. A number of computational models of signaling pathways have been described (Bhalla et al., 2002; Hoffman, et al., 2005).

Many toxicity pathways converge on the same targets. These targets can serve as biomarkers of effect - they are not indicative of exposure to a specific chemical, but rather indicate that toxic effects are or may soon be underway in the organism. Some examples include: caspase cleavage (marker of apoptosis), activation of NF-κB (non-specific marker for cancer, inflammation, auto-immune disorders, etc.), increases in 8-oxo-dG (non-specific marker of oxidative stress), and DNA strand breaks (marker of genotoxicity, i.e. as measured by the comet assay). The level of 8-oxo-dG can be measured using immunofluorescence in whole cells or tissue slices (Machella, et al. 2005), by mass spectrometry in hydrolyzed DNA (Singh, et al. 2009) and urine samples (Mesaros, et al. 2012), by ELISA in serum, urine, and saliva samples[8], or by HPLC coupled with ultraviolet absorption and electrochemical detectors (HPLC/UV/ECD)

[8] http://www.rndsystems.com/Products/4370-096-k/

(Prahalad, et al. 2000). The diversity of samples that are suitable for analysis frees sample choice to allow consideration of multiple factors, such as accessibility, cost, and sensitivity to be taken into account. The variety of platforms enables labs to use existing equipment to carry out the study, so if they don't own an EC detector they can switch to mass spectrometry instead. And finally, since many different processes lead to an increase in oxidative stress, and hence elevated concentrations of 8-oxo-dG, this can be used as a first-line assay for prioritization and an estimation of relative potency. The downside of generic assays such as this is that same lack of specificity. Experiments must be carefully controlled for confounding variables, such as vehicle (like dissolving the study chemical in DMSO), study-induced stress, and known sources of reactive oxygen species. So long as results are placed in the proper context, biomarkers of effect can be a powerful tool for identifying toxicity pathways and establishing a mechanism of action that connects exposure to apical effect.

Many of these models depend heavily on *in vitro* and animal *in vivo* data to provide the quantitative information about these key events (Shah and Wambaugh, 2010). This approach can sometimes be complicated due to the body's ability to maintain homeostasis, and tolerate relatively large perturbations. The challenge to modelers is to find the minimal level of perturbation that is necessary to significantly increase the likelihood of an apical effect, and to characterize the dose-response relationship at doses around this threshold level. For human health risk assessment, the reliance on animal data exclusively to parameterize these models can lead to problems when making quantitative predictions. For this purpose, animal studies should include a biomarker discovery component whereby biomarkers from accessible matrices

(e.g., blood, urine) are quantitatively linked to these key events. These biomarkers can be defined as bioindicators to indicate this quantitative linkage to a key event. The bioindicators are now available for human biomonitoring and epidemiological studies to adjust the computational models for quantitative differences between animals and humans. Since these bioindicators are being used to link toxicity pathways to adverse outcomes, there is no need to perform this action for all chemicals. Once the quantitative relationship between the toxicity pathway perturbation and the adverse outcome has been established, predictions for new chemicals can be made based on the toxicity pathway perturbation alone.

Cheminformatics-based Modeling

While exposure, dosimetry, and biological models may be the most valuable and informative way to work with biomarker data, when considering the large numbers of chemicals and the wide spectrum of ADME or other biologically-based characteristics that each chemical exhibits, the amount of time and effort required to develop such models can be prohibitive. Cheminformatics techniques are typically much less intensive to apply, but still provide key insights into the nature of biomarkers. Borrowing from "chemical space" concepts (Oprea and Gottfries 2001; Kirkpatrick and Ellis, 2004; Dobson, 2004; Larsson et al., 2007), the domain of potential biomarkers can be defined as the set of all stable and possible Stoichiometric combinations of atoms, electrons and neutrons that give rise to a pharmacokinetic signature and/or presence that is detectable in biological media (e.g., blood, urine, feces, breath). Complete characterization and identification of all possible biomarkers and their associated properties for the wide spectrum of exposure scenarios in this chemical

domain or space, even with HTS methods, is a costly and time consuming endeavor. Current cheminformatic techniques can be utilized to explore this large chemical space more efficiently and effectively while also having the potential to rapidly inform and update more data-intensive models in the absence of available pharmacokinetic data.

With this perspective, it can be advantageous to visualize the mapped chemicals of interest (e.g., potential biomarkers, NHANES chemicals, relevant chemical-specific PBPK models) in a manner that compares qualitative characteristics or molecular descriptors universally (i.e., globally) or, at a minimum, relative to each other (i.e., locally). Navigation within chemical space requires visualization of a plurality of molecular entities of interest within the molecular descriptor dimensions of relevance (i.e. in a visually tractable reduced principal component representation). Visualization approaches, such as 3-dimensional principal component analysis (3D-PCA) plots, in support of chemographic exploration provide rapid visual insights into the similarity/dissimilarity of chemical biomarkers based on the relative mapped positions of one molecular entity's structure-based/biologically-based properties with relation to another "neighboring" entity in Euclidean space (Oprea and Gottfries, 2001; Oprea et al., 2002). The motivation for mapping chemical properties is largely driven by the discovery of chemicals that exhibit similar behavior or properties, abiotic or biologically-mediated. This discovery aspect has apparent implications in both fields of pharmaceutical (Lipinski et al., 1997; Shoichet 2004; Obach et al., 2008; Kar and Roy, 2013; Barker et al., 2013) and material sciences (Broderick et al., 2008; Martin et al., 2011). Within the field of exposure sciences, the chemical mapping of biomarker properties can lead to significant insights, including but not limited to:

i) Determining the suitability of using existing computational models for specific chemicals to identify new biomarkers (i.e. domain of applicability);

ii) Characterizing chemical space of these models in relation to existing exposure or hazard databases to improve identification of chemical surrogates for specific parameters (i.e. analog proxies, such as a identifying benzene rather than perchloroethylene as a surrogate for toluene), and chemical space data gap analysis); and

iii) Elucidating qualitative behaviors in biomarkers to improve future or existing study designs.

As a case study, a survey of the chemical landscape for existing PBPK models was conducted to provide qualitative insights into the development of dosimetry models for chemicals with little or no data. By mapping both chemical structure and property domains to existing PBPK models, the identification of potential biomarker knowledge domains can be elucidated within available chemical databases. Utilizing the workflow outlined in Figure 5, a retrospective literature survey (up to 2010) indicates that there were approximately 480 PBPK-related publications (review articles were omitted). Among these publications, PBPK models existed for approximately 100 unique chemical entities. A principal component analysis (PDA) using the web-accessible ChemGPS-NP protocol (Larrson et al., 2007; Rosen et al., 2009) was performed on the current physicochemical landscape upon which these PBPK models and three large open-access national databases, including NHANES IV[9] (2003-2004), the U.S. Department of

[9] http://www.cdc.gov/exposurereport/pdf/NER_Chemical_List.pdf

Agriculture's Pesticide Data Program[10] (USDA-PDP), and the U.S. Environmental Protection Agency's ToxCast™ Phase 1[11]. Additional molecular descriptors were evaluated for each set, including CACO-2, MDCK and blood brain barrier permeability based on Jorgenson's solvent-accessible surface area calculations as implemented within QikProp (Duffy and Jorgensen, 2000; Jorgensen and Duffy, 2000; 2002). Due to lack of parameterization for evaluating metal and metallo-organic properties, only organic compounds were retained in this analysis. The impact of such omissions, however, was found to be relatively negligible — of the 276 chemicals in the NHANES IV dataset, 93% are classified as organics while 7% are metals and metallo-organics. For the current analysis, 256, 280, and 320 chemicals were included from NHANES IV, USDA-PDP and ToxCast™ Phase I databases, respectively.

From the ChemGPS-NP mapping, the resulting property-based projections of the chemicals from different databases can be compared and easily interpreted together with their respective trends and clusters. Our analysis finds that both USDA-PDP and ToxCast™ Phase 1 databases share roughly 70% of the same chemical space while approximately 40% of the same chemicals are shared between NHANES and ToxCast™ Phase 1 databases. The larger overlap between USDA-PDP and ToxCast™ chemicals is largely attributed to the similar interests within these datasets (i.e., pesticides). A similar analysis shows that the PBPK domain found within our literature survey exhibits high overlap with the existing NHANES IV chemical domain (Figure 6).

[10]http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateC&navID=PesticideDataProgram&rightNav1=PesticideDataProgram&topNav=&leftNav=ScienceandLaboratories&page=PesticideDataProgram&resultType

[11] http://www.epa.gov/ncct/dsstox/sdf_toxcst.html

Surveying the landscape of evaluated molecular properties, chemicals within the PBPK domain were found to have higher average cell permeabilities (CACO-2 and MDCK) and blood-brain-barrier transfer coefficients than chemicals within either the USDA-PDP or ToxCast^{TM} Phase 1 databases. The result is consistent with our observation that chemicals in the PBPK domain have, on average, the lowest molecular weights and solvent-accessible surface area, which is directly proportional to these calculated properties. Similarity of chemical features within both NHANES and PBPK domains suggests that PBPK models already exist for many of the NHANES chemicals and/or that existing PBPK models may be very similar to a subset of the NHANES chemicals. In the latter case, the PBPK models may be modified for use in model studies based on the close similarity of the NHANES chemicals to the chemical biomarker of interest. This finding, however, does not signify that existing PBPK models in their unmodified state are necessarily suitable for describing the dose-biomarker relationships for these NHANES chemicals. Most PBPK models were developed and calibrated using animal kinetic data, and they were used to predict concentrations of toxic moiety at the target tissue of interest within each study objective. Human biomarkers such as those in NHANES, however, are seldom collected from target tissues. The particular choice of biomarkers is primarily based on specificity, sensitivity, accessibility, and available methods of analysis. Hence, biomarkers are mostly metabolites in urine, which are generally more hydrophilic and less toxic, and thus, not specifically predicted in most PBPK models. On the other hand, adequate human biomarker (e.g., repeated blood samples) and exposure data may be used to evaluate, validate and/or recalibrate human PBPK models subsequently scaled from existing animal models.

Interpreting available biomarker data requires both pharmacokinetic and chemical-specific knowledge of the parent molecule and/or its subsequent metabolites within an accessible biological media. The particular choice of biomarkers through specific elimination pathways can influence study results, including derived exposure estimates. Determining the major elimination pathway a priori can aid in devising relevant experimental designs that properly detect biomarkers within their respective biological media. One method of determining potential elimination pathways would invoke a simple generalized form of chemical-specific PBPK models. A distributional analysis which determines the potential effects of pharmacokinetic distribution within the body based on its chemical properties (e.g., partitioning) would represent a first step in approximating the elimination pathways of various exogenous chemicals. Without consideration for metabolism, potential chemical biomarkers in such a simple analysis would reveal relative percentage across various elimination routes within the body – i.e., breath, urine and feces. As more information regarding metabolism is provided, a better pharmacokinetic approximation to our non-metabolized chemical biomarker assumption would be incorporated into this framework.

To illustrate this case, the generalized fugacity based PBPK model of Cahill and his colleagues (2003) was implemented for all organic chemicals within the three aforementioned databases, as well as the identified unique chemicals across the PBPK literature domain. The model is parameterized for a 70 Kg male adult and it requires chemical-specific properties including molar mass (*ad hoc* assumption of 300 g/mol average weight), molar volume (300 cm^3), octanol-water partition coefficient ($K_{o/w}$), air-water partition coefficient ($K_{a/w}$), as well as specific

reaction rate parameters for both liver and intestine. This model is amenable to a variety of exposure routes including oral, inhalation and IV injection. The model has been translated to a spreadsheet-based Microsoft Excel platform[12]. In our case, a 10 day simulation was run for each chemical as 10 mM single dose intravenous (IV) injections, while observing the relative body burden and fate of each chemical. The simulated relative loss mechanisms of each chemical via feces, urine and air (breath) pathways was further analyzed for the NHANES chemicals and average values are reported for the classification given by the NHANES IV report. Available chemical-specific data (e.g., $K_{o/w}$, $K_{a/w}$) required for the model were obtained from EPISuite calculations (EPA 2012).

In Figure 7, results for chemicals derived from each dataset are presented in a PBPK model "inspired" elimination hypersurface that is projected onto the observed range of log $K_{o/w}$ and log $K_{a/w}$ values. The additional database developed by Obach and colleagues (2008) containing human IV pharmacokinetic data on 670 drugs is displayed for reference. The overlay of brown, yellow and blue colored regions represents the relative percentage of fecal, urinary and breath elimination pathways for a given value of partition coefficients, respectively, that exist for our defined "average" molecule (i.e., *ad hoc* assumptions for molar mass and volume values). The advantage of such a representation allows for rapid and succinct conveyance of the relative location in excreta space for a large number of chemicals.

[12] http://www.trentu.ca/academic/aminss/envmodel/models/PBPK.html

The hypersurface analysis suggests that the trends as previously observed in the PDA are similar - i.e., large overlap and similarity between USDA-PDP and Toxcast^{TM} Phase 1 as well as closer proximity between NHANES and existing PBPK model domains. Plotted centroid values illustrate central tendencies observed in the databases and captured in our average molecular property survey of each database. Furthermore, this analysis allows for the identification of potential biomarker matrices. Intuitively, octanol-water and air-water partition coefficients are a measure of a chemical's relative lipophilicity and volatility, respectively. In general, low volatile ($\downarrow K_{a/w}$) and highly lipophilic ($\uparrow K_{o/w}$) chemicals tend to be eliminated via a fecal route while high volatile ($\uparrow K_{a/w}$) chemicals tend to be eliminated via a breath or air route. The former case is typified for a high proportion of USDA-PDP and ToxCast^{TM} Phase 1 chemicals. The latter case is illustrated for a set of NHANES and PBPK model domain chemicals in Figure 7. Not too surprisingly, chemicals within the drug-centric database of Obach and colleagues (2008) have on average the lowest volatility relative to the other chemical domains where lower volatility/increased solubility in drugs has the potential to lead to increased therapeutic bioavailability - a desired outcome in drug discovery. In contrast, potential biomarkers that exhibit increased bioavailability might be less desirable especially if there is an associated adverse outcome or effect at some observed target tissue dose level.

Besides the potential identification of chemicals within a particular biomarker matrix, the analysis of the relative loss mechanisms can also give particular insight into chemical classification of biomarkers. Further analysis yields results given in Figure 8 where chemical

class average relative elimination for each route over the 10 day simulation is represented. A simple chemical partition decision tree classifier can be used to discern average behaviors observed based on the NHANES chemicals. In this case, greater than 90% and less than 10% urine fractions were modeled with misclassification rates of 3.3% and 2.9%, respectively. The analysis further corroborates the hypersurface observations where the class of volatile organic compounds is shown to undergo on 75% "average" elimination via breath. Since breath and blood concentrations of some chemicals have been shown to be correlated (Narasimhan et al., 2001; Gainsford et al., 2006), this observation could present an alternative to using traditional blood sampling techniques for other highly volatile classes of compounds. Alternatively, low volatility and highly lipophilic compounds could be potentially underestimated in current biomarker analyses especially if a large portion of the fraction eliminated occurs through non-urinary routes (i.e., fecal) as some chemicals in these simulations suggest. However, the uncertainty due to metabolism presents the largest confounder in this analysis. Conceptually, metabolism seeks to lower lipophilicity and volatility through conjugation, oxidation or other mechanisms thereby making the parent chemical more "soluble". Inclusion of these effects would essentially shift the relative position of each chemical to the lower left hand quadrant of the hypersurface and potentially into the urinary elimination phase. Therefore, while the utility of these concepts can be utilized to ascertain insights with the biomarker chemical space domain, caution should always be taken when considering the approximations incurred.

The value of these applied cheminformatic-based techniques is in what they provide: effective guidance towards better approximations to available models, and predictive methods for

making rapid assessments that inform existing and future exposure biomarker studies. These methods provide a provisional means to start exploring poorly sampled biomarker space and to devise methods upon which to inform and increase our knowledge in these areas and identify data and/or knowledge gaps through the use of chemical surrogates and or surrogate dosimetry models. Identification of overlaps in chemical databases for biomarkers and non-biomarkers could potentially inform the development of knowledge bases that will make data transfer more efficient between both modelers and experimentalists. Finally, understanding the chemical landscape of exposure biomarkers can also provide a first step analysis in identifying potential biomarker elimination profiles as well as the accessible target biological media necessary for developing new analytical methods.

Conclusions

Computational Toxicology provides important tools to extend our current understanding of biomarker measurements and enable the discovery of new biomarkers. Snapshots of biomarker concentration, along with knowledge of exposure, ADME, and toxic responses, can provide insights as to how exposures to molecular entities contribute to elevated health risks. Biomarkers must be merged with other available tools and data to move towards a systemic understanding of exposure-health effects linkage. Computational toxicology, with its breadth of research, is well suited for facilitating this movement, and will foster improved use of biomarker data in future human health assessments.

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Figure 1. Time course of simulated chemical X in both blood and urine.

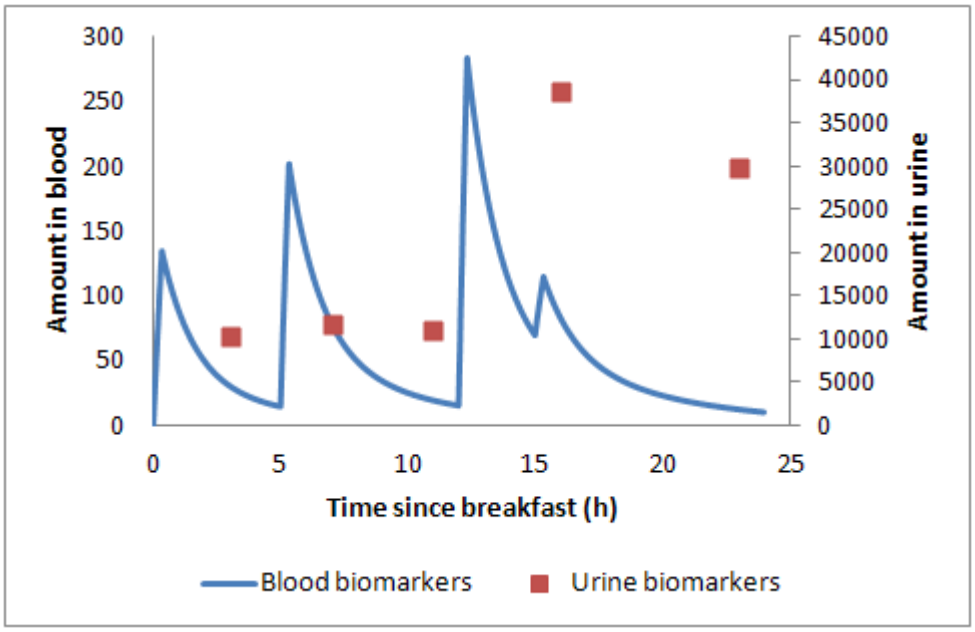


Figure 2. Differences between animal toxicity studies and human biomonitoring studies.

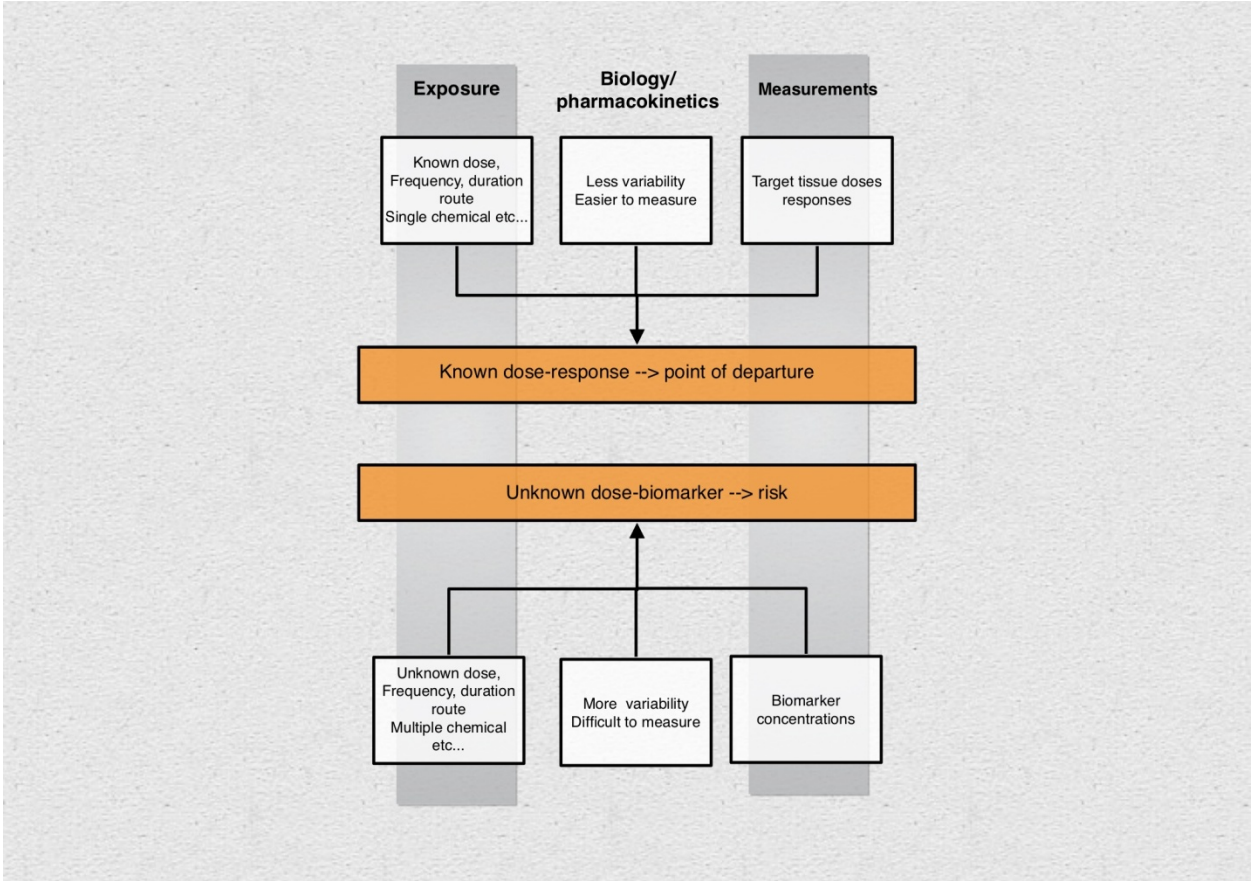


Figure 3. Workflow for using U.S. EPA's PROcEED tool for performing probabilistic reverse dosimetry

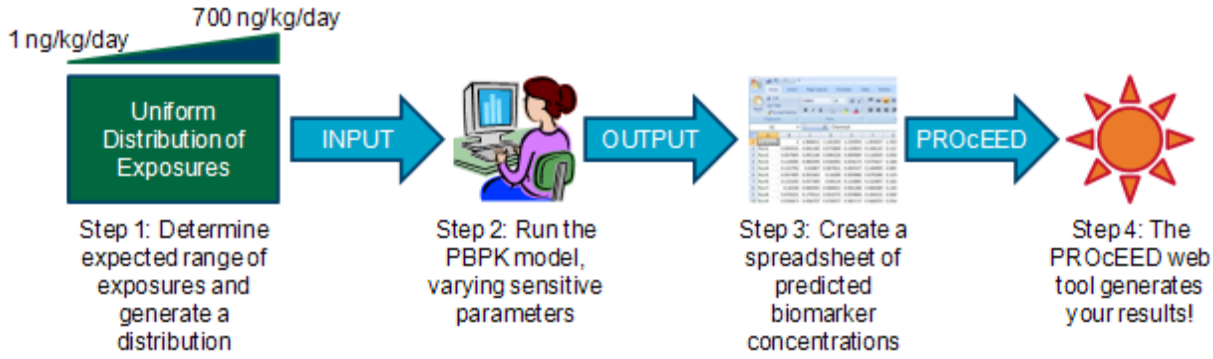


Figure 4. PROcEED reverse dosimetry estimated daily intake doses (ng/kg/day) for perchlorate based on NHANES 2001-2002 urinary perchlorate concentrations.

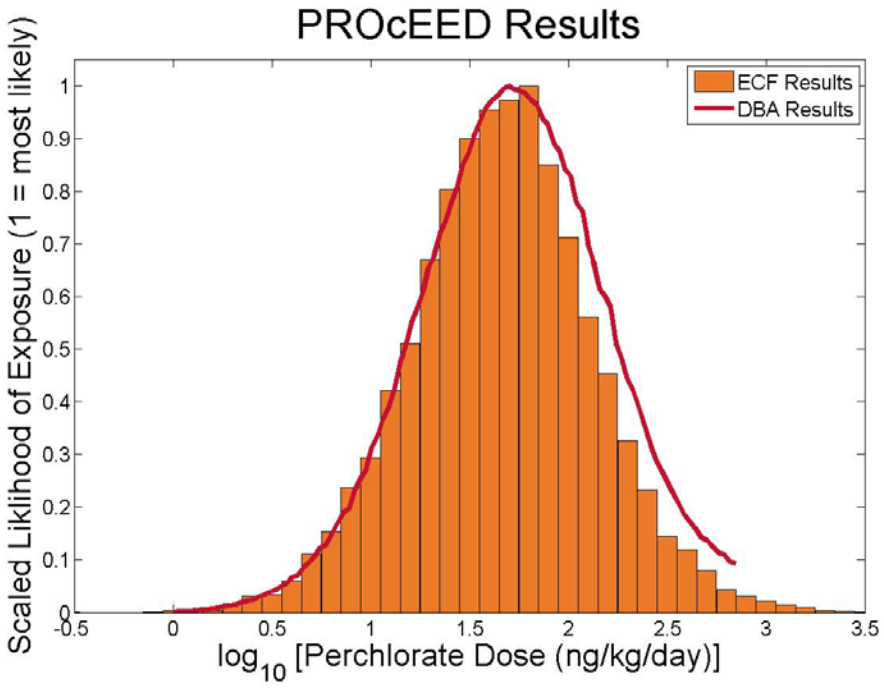
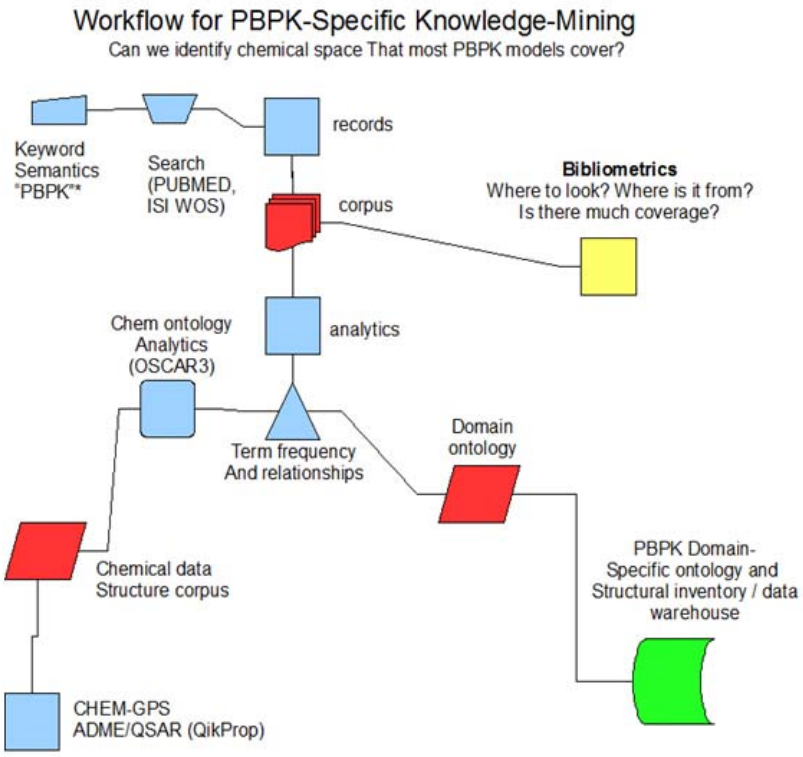


Figure 5. A workflow for surveying chemical landscape for the existing PBPK models.



*= ("pbpk"[Title/Abstract] OR "pbpk/pd model"[Title/Abstract] OR "pbpk/pd modeling"[Title/Abstract] OR "pbpk/pd models"[Title/Abstract] OR "pbpk model"[Title/Abstract]) AND (human OR animal) NOT review

Figure 6. Overlap of chemicals within 3 publicly available databases and literature PBPK chemical-specific PBPK models through 2010. Red dots: PBPK model chemicals, yellow dots: NHANES chemicals; purple dots: USDA-PDP chemicals; green dots: ToxCastTM Phase 1 chemicals. The first 4 dimensions of the PCA are plotted where PC1 accounts for molecular size, shape and polarizability; PC2 accounts for aromatic and conjugation related properties; PC3 accounts for lipophilicity, polarity, and H-bond capacity; and PC4 describes molecular flexibility and rigidity within the ChemGPS-NP analysis.

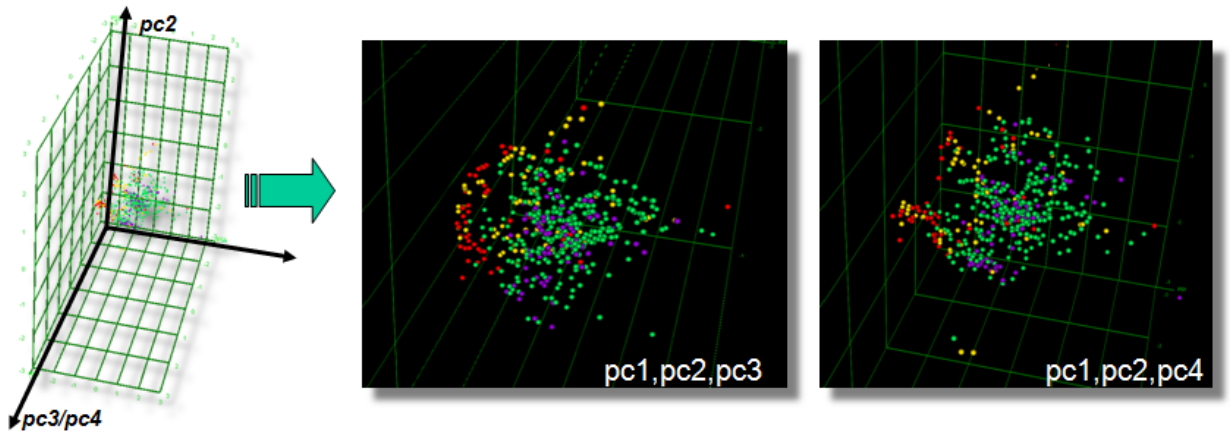


Figure 7. PBPK-inspired hypersurface analysis for overlaying NHANES, ToxCastTM Phase 1, and USDA-PDP databases, PBPK model domains and 670 drugs from Obach and colleagues (2008). Centroid values for each database domain are depicted as large filled and unfilled circles colored according to labels.

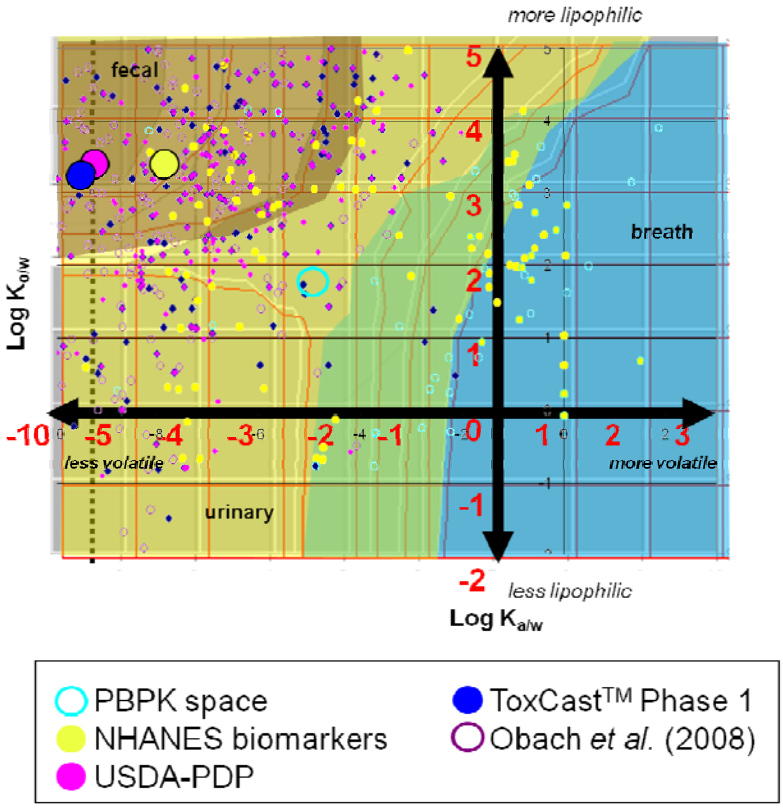


Figure 8. Simulated NHANES IV predicted fraction of elimination by route after 10 days based on chemical classes within NHANES IV report and the derived decision tree classifier for chemicals with >90% and <10% urine elimination relative to normalized total values.

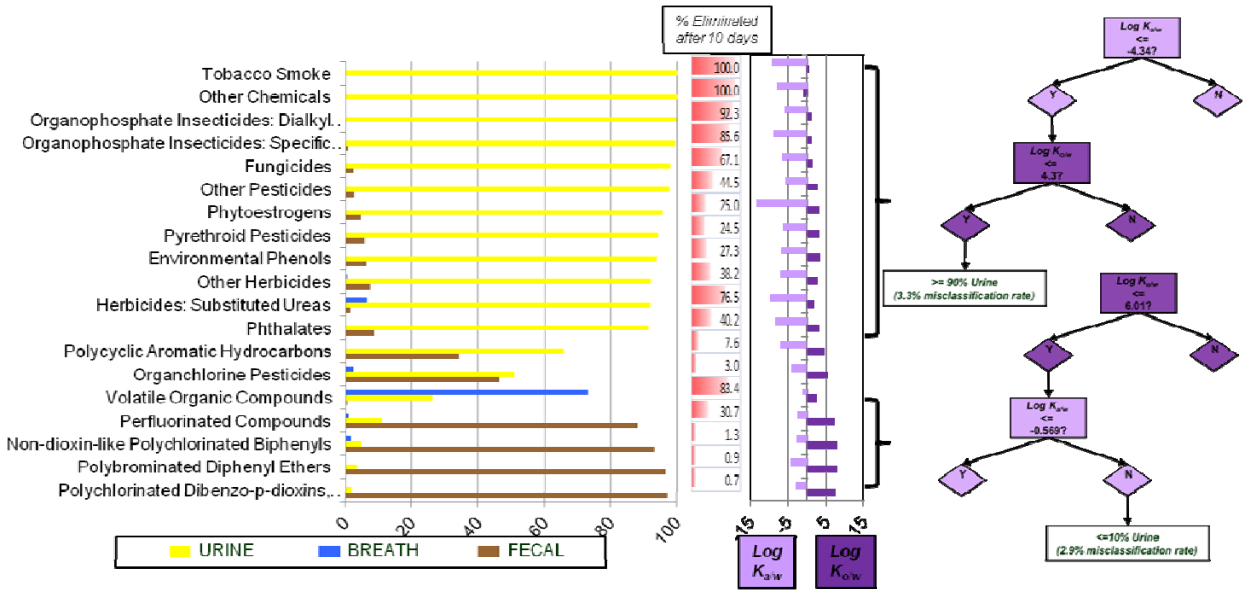


Table 1. Exposure scenarios, biomarker sampling parameters, and metabolism rates between two individuals.

	Intake dose	Time of exposure	Time of sampling	Urine volume	Metabolism rate Y to M1	Metabolism rate M1 to M2
1	A > B	Same	Same	Same	Same	Same
2	Same	Same	Same	Same	A > B	Same
3	Same	Same	Same	Same	Same	A > B
4	Same	Same	Same	A > B	Same	Same
5	Different	Different	Different	Different	Different	Different