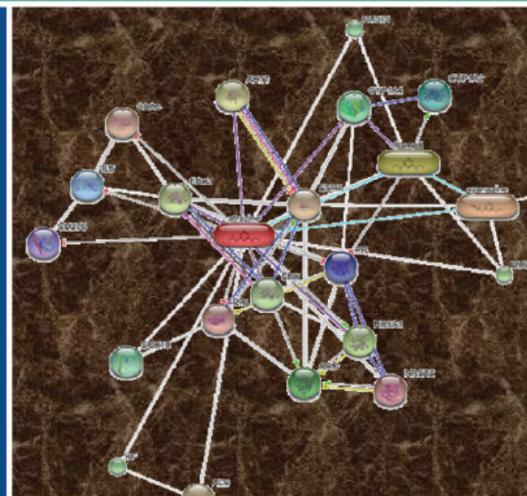


Biomonitoring — An Exposure Science Tool for Exposure and Risk Assessment

DEVELOPED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL EXPOSURE RESEARCH LABORATORY
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CONTRIBUTORS

Cecilia Tan, Curtis Dary, Daniel Chang, Elin Ulrich, Jeanette Van Emon,
Jianping Xue, Joachim Pleil, John Kenneke, Jon Sobus, Linda Sheldon, Marsha Morgan,
Rocky Goldsmith, Rogelio Tornero-Velez, Ross Highsmith, Roy Fortmann,
Tim Collette, Valerie Zartarian (EPA/ORD/NERL)

PRIMARY CONTACT

Cecilia Tan

National Exposure Research Laboratory,
109 T.W. Alexander Dr., Research Triangle Park, NC 27711

DISCLAIMER

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Abstract

Biomonitoring studies of environmental stressors are useful for confirming exposures, estimating dose levels, and evaluating human health risks. However, the complexities of exposure-biomarker and biomarker-response relationships have limited the use of biomarkers in exposure science studies. In this document, an updated source-to-outcome continuum is presented to better define biomarkers as tools for human health research; specific attention is given to biomarker applications in exposure research. This continuum links exposure sources and health outcomes using a compilation of measurements, mathematical models, and model

estimates. A tiered approach to biomonitoring analyses is presented, based on this continuum, to categorize the uses of biomonitoring data given various research objectives and the availability of specific measurements and models. Tools that can be used to infer critical model parameters and model estimates (when they are unavailable) also are discussed to improve biomarker utilization for exposure and risk assessments. Finally, frequently encountered complications in biomonitoring studies are discussed, along with suggestions to address these challenges.

1.0 Introduction

Human exposure assessment for environmental chemicals traditionally has focused on identifying sources, determining chemical fate and transport, and quantifying the resulting microenvironmental concentrations in media with which humans come in contact. Exposure assessment provides information for use in risk assessment on the magnitude, frequency, and duration of the intersection between a stressor and a receptor. During the assessment process, uncertainties can arise from numerous sources, including extrapolations from external concentration to internal dose. Some of these uncertainties may be reduced by directly measuring chemicals and/or their metabolites in biological samples through biomonitoring studies. Historically, biomonitoring studies have been used to confirm environmental exposures. When carefully planned, these studies have the potential to provide estimates of internal dose or evaluate possible health risks. The full potential of biomonitoring, however, is yet to be realized because obtaining the maximum possible value from biomonitoring requires information, which is often lacking, about exposure, toxicology, pharmacokinetics, and epidemiology.¹

In 2006, the National Research Council (NRC) of the National Academy of Sciences (NAS) conducted an independent study to review the current practices of interpretation and uses of conventional biomonitoring data (i.e., chemicals, their metabolites in human tissues/specimens). In its report, the NRC identified data gaps when considering biomarkers for specific applications, such as risk assessment. Specifically, the NRC recommended the need to “develop biomonitoring-based epidemiologic, toxicologic, and exposure-assessment investigations and public-health surveillance to interpret the risks posed by low-level exposure to environmental chemicals.”

¹ NRC (National Research Council) (2006). Human Biomonitoring for Environmental Chemicals. National Research Council Committee on Human Biomonitoring for Environmental Toxicants. National Academies Press, Washington, DC.

In response to the NRC’s recommendations, this document outlines the research strategies proposed by researchers in the U.S. Environmental Protection Agency (EPA) National Exposure Research Laboratory (NERL) to generate data and develop/refine tools for improving the use and interpretation of biomonitoring data in human exposure and risk assessment. These research strategies will address the following key science questions.

1. What are the key elements of a source-to-outcome continuum that includes biomarkers as a critical link for exposure and health effects research?
2. How do we interpret biomonitoring data to improve exposure and risk assessments using the methods, measurements, and models developed or in use by the research community?
3. How do we develop and incorporate new biomarkers and apply emerging technologies to better assess human exposure and resulting health effects?

The enhanced science and other products from this research program will lead to the following expected outcomes.

1. A framework that provides guidance for assessing exposures and/or health risks using biomonitoring data.
2. Innovative application of emerging/evolving technologies for determining and analyzing biomarkers of exposure for small molecules.
3. Incorporation of biomonitoring data and relevant exposure, pharmacokinetic, and toxicological data/tools for informing the Agency’s risk assessment and management decisions for human and wildlife health.

2.0 A Source-To-Outcome Continuum for Human Health Research ²

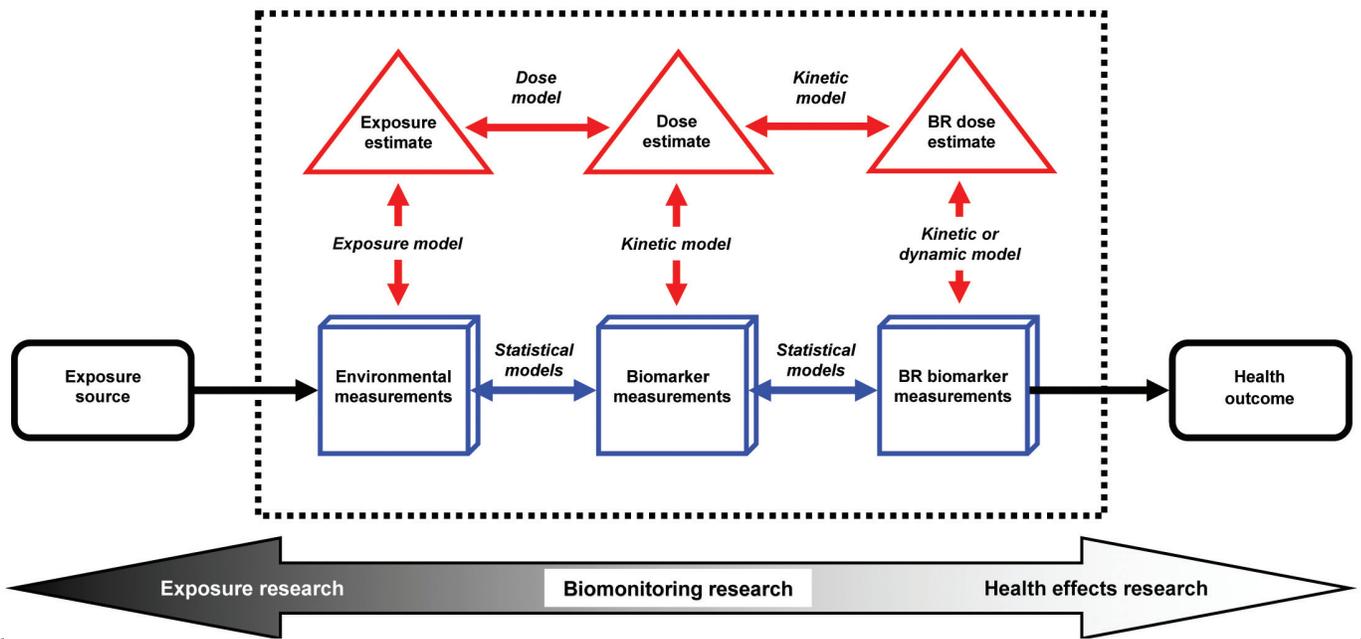
In this document, an updated source-to-outcome continuum is presented to better define biomarkers as tools for human health research. The components and linkages of this updated continuum (Figure 1) highlight specific research needs (i.e., measurements, models, estimated values) for observational studies of human populations. The left side highlights traditional components of exposure research whereas the right side highlights contemporary components of health effects research. Biomarker measurements are central to the continuum and, therefore, link the exposure and health effects components. The following sections define individual components of the continuum and describe how they can be used to answer exposure- and risk-based questions for human health research.

The source-to-outcome continuum for human health research (shown in Figure 1) contains the following eleven components (not including source and outcome).

1. *Environmental measurements* are observed stressors in environmental media that reflect (either directly or indirectly) an exposure source. Although stressors can be biological (e.g., bacteria), physical (e.g., radiation), or even psychosocial (e.g., stress), chemical stressors in the environment are the focus of this discussion. Examples include chemical concentrations in foods (chemical mass per unit food mass), in drinking water (chemical mass per unit water volume), in consumer products (chemical mass per item), and in outdoor and indoor air (chemical mass per unit air volume).
2. *Exposure models* generate exposure estimates by mathematically combining environmental measurements with human activity observations. Example human activities in exposure models include eating food, drinking water, applying consumer products, and spending time indoors versus outdoors.
3. An *exposure estimate* is the predicted amount of chemical (total mass) that comes into contact with a human. These estimates can be route-specific (e.g., inhalation exposure, ingestion exposure, dermal exposure) or summed across all routes (i.e., aggregate exposure). An example exposure estimate from the diet is food concentration ($\mu\text{g}/\text{mg}$ food) \times food consumption (mg food/meal) = dietary exposure ($\mu\text{g}/\text{meal}$). Exposure levels generally are fixed in animal studies (e.g., $\text{mg}/\text{kg}/\text{day}$, referred to as “external dose”) but are inferred in observational exposure studies using environmental measurements and human activity observations.
4. *Dose models* generate dose estimates by mathematically combining exposure estimates with parameters that describe chemical movement into the body from the site(s) of contact. For inhalation, ingestion, and dermal exposure, chemical movement into the body (i.e., absorption) often occurs through the lungs, gut, and skin, respectively. Although exposure and dose models are shown separately in Figure 1, parameters describing chemical exposure and absorption often are included in combined exposure-dose models.
5. A *dose estimate* is the amount of a chemical (total mass) that enters the body. In health effects studies, dose levels are unambiguous because conditions are generally deliberate and well controlled. Furthermore, dose levels in animal studies are adjusted by body weight (e.g., $\mu\text{g}/\text{kg}$) to allow inter- and intraspecies comparisons and are a basis for toxicity reference values (e.g., reference dose [RfD]). Alternatively, dose estimates from observational exposure studies rely on environmental measurements, human activity observations, and absorption predictions and are, therefore, subject to uncertainty.
6. *Kinetic models* mathematically describe the movement of a chemical through the body; that is, the chemical’s distribution to various tissues, metabolism by various processes, and ultimate elimination from the body. (Absorption parameters from dose models frequently are included in kinetic models to simultaneously address absorption, distribution, metabolism, and elimination). Parameters for these models generally are estimated using *in vitro* metabolism experiments, *in vitro* kinetic studies with animals, and controlled human exposure studies. Parameterized models from these experiments can be applied in observational exposure studies to predict tissue/fluid levels of chemicals and metabolites following exposure events.
7. *Biomarker measurements* are observations of chemicals, chemical metabolites, and target molecules in media, such as blood, urine, breath, fingernails, hair, milk, and feces. These observations can reflect exposure events (biomarkers of exposure), health status (biomarkers of effect), and systemic functions (biomarkers of susceptibility). However, discussions herein pertain strictly to biomarkers of exposure. As such, example biomarkers include native (unmetabolized) chemicals, phase-I metabolites (e.g., oxidized, reduced, or hydrolyzed chemicals), and phase-II metabolites (e.g., glutathione-, glucuronic acid-, and sulfate-conjugated chemicals).

² Context in this section has been summarized in a published article: Sobus et al. (2011). A biomonitoring framework to support exposure and risk assessments. *Science of the Total Environment*, 409(22):4875-4884.

Components of human health research



Symbol	Key	Parameter	Definition
	Estimated value	1) Exposure estimate 2) Dose estimate 3) BR dose estimate	1) Estimated level of human contact with an analyte 2) Estimated level of analyte that enters a human 3) Estimated level of analyte that reaches a target within a human
	Measured value	1) Environmental measurement 2) Biomarker measurement 3) BR biomarker measurement	1) Measured level of analyte in environmental media that reflects an exposure source 2) Measured level of analyte in biological media that reflects a dose 3) Measured level of analyte in biological media that reflects a BR dose
	Empirical model	1) Statistical model (blue)	1) Model that evaluates observed variables for hypothesis testing
	Mechanistic model	2) Exposure model (red) 3) Dose model (red) 4) Kinetic model (red) 5) Dynamic model (red)	2) Model that estimates exposure using environmental measurements and human activities 3) Model that estimates how much analyte enters a human 4) Models that describe how an analyte moves through and is removed from a human 5) Model that describes the effect of an analyte on the human body

Figure 1. A source-to-outcome continuum for human health research.

8. A *biologically relevant* (BR) dose estimate is the amount of the dose that reaches a target and is available to contribute to health impacts. For example, the BR dose of a neurotoxic chemical may be the amount of chemical or metabolite that reaches the brain. For a genotoxic chemical, the BR dose may be the amount of chemical or metabolite that interacts with genetic material. These values can be determined directly in some animal studies, generally via biopsies of target tissues. However, BR dose levels in observational exposure studies are estimated using kinetic models and rely on preceding exposure and dose estimates.
 9. *Dynamic models* mathematically describe the impacts of the BR dose on biological systems. For example, dynamic models for neurotoxic chemicals may describe the rates of enzyme inhibition in the brain, whereas those for genotoxic chemicals may describe the rates of DNA damage and repair. In general, these models are parameterized using data from *in vitro* experiments and *in vitro* studies with animals. Combined kinetic and dynamic parameter estimates then can be applied in observational exposure studies to predict systemic changes as functions of the estimated BR dose.
 10. *BR biomarker measurements* are observations of analytes in biological media that reflect (directly or indirectly) the BR dose of a chemical or group of chemicals. BR biomarkers can be chemical metabolites, chemically altered molecules (e.g., adducts of reactive electrophiles), or non specific markers of systemic processes (e.g., levels of hormones, antibodies, or gene expression). They differ from “biomarkers of effect” in that they are not strictly markers of impaired function or disease endpoints. That is, they may be, but are not required to be, associated with key events in a disease process. For example, BR biomarkers for genotoxic electrophiles can be markers of genetic damage (direct markers) or products of reactions with blood nucleophiles (indirect markers).
 11. *Statistical models* are empirical models that compare observed random variables for hypothesis testing. For example, statistical models can evaluate associations between environmental and biomarker measurements of the same chemical and between biomarker measurements and BR biomarker measurements. Statistical models also can evaluate the effects on these relationships of confounding variables, such as age, gender, human activities, health status, and time (e.g., when measurements are made). Therefore, statistical models are used to attribute measurement variation to explanatory factors in observational human health studies.
 12. Figure 1 shows that components of the source-to-outcome continuum align along two planes: (1) a plane of measured values (i.e., environmental, biomarker, and BR biomarker measurements) that are shown with blue boxes; and (2) a plane of estimated values (i.e., exposure, dose, and BR dose estimates) that are shown with red triangles. Exposure, dose, and BR dose can be linked to health outcome in *controlled* studies, yet all of these values are estimated in *observational* studies. As such, these values rely on measurements, activity observations, and model parameter estimates and are, therefore, subject to uncertainty. Biomarker measurements, which are at the center of the continuum, can reduce uncertainties by answering specific exposure- and risk-based questions. The following section demonstrates these uses of biomonitoring via the following five research tiers.
 - Tier 1: Biomonitoring for exposure surveillance
 - Tier 2: Biomonitoring to support exposure assessment
 - Tier 3: Biomonitoring to support risk assessment
 - Tier 4: Biomonitoring for exposure and risk assessment
 - Tier 5: Biomonitoring to advance exposure and risk assessments
- Tier 1 considers only biomarker measurements, and subsequent tiers consider additional measurements, models, and estimated values. Example biomarkers for each tier are assumed to be measurable using reliable sampling and analytical methods and to reflect exposure to environmental chemicals (a discussion of these assumptions is given in section 5). Simple theoretical examples are given for the biomonitoring tiers to demonstrate how biomarker data can be used to answer important exposure- and risk-based questions. Theoretical examples are given, rather than results from published studies, to enable continuity from one tier to the next and to simplify the interpretation and discussion.

3.0 Biomonitoring Tiers for Exposure and Health Research³

3.1 Tier 1: Biomonitoring for exposure surveillance

Tier 1 analyses of biomarker data aim to answer one or more of the following questions for exposure surveillance.

1. Who is exposed?
2. What are the exposure trends?
3. Which chemicals should be prioritized for higher tier analyses?

Figure 2 is an adaptation of the source-to-outcome continuum that shows biomarker measurements as the only requirement for a tier 1 analysis. Specifically, cross-sectional biomarker measurements are used in tier 1 analyses for evaluating exposures across populations, and longitudinal biomarker measurements are used for evaluating exposure trends within

³ Context in this section has been summarized in a published article: Sobus et al. (2011). A biomonitoring framework to support exposure and risk assessments. *Science of the Total Environment*, 409(22):4875-4884.

a population. To demonstrate these uses, two theoretical examples are given in Figure 2. Example 1 displays cumulative distributions of biomarker levels for two groups (a cross-sectional analysis), and example 2 shows average biomarker levels for one group as a function of time (a longitudinal analysis).

In example 1, the two distributions represent biomarker measurements that have been separated into groups for hypothesis testing. Example groups include those separated by gender (i.e., male versus female), geographic location (i.e., location 1 versus location 2), age (e.g., < 18 years old versus ≥ 18 years old), source impact (e.g., product users vs. nonusers), and health status (e.g., healthy versus health impaired). Observed differences between grouped measurements indicate an effect of the grouping variable on biomarker levels, and suggest exposure differences between the groups. This type of tier 1 cross-sectional analysis is used for identifying populations with elevated exposure levels

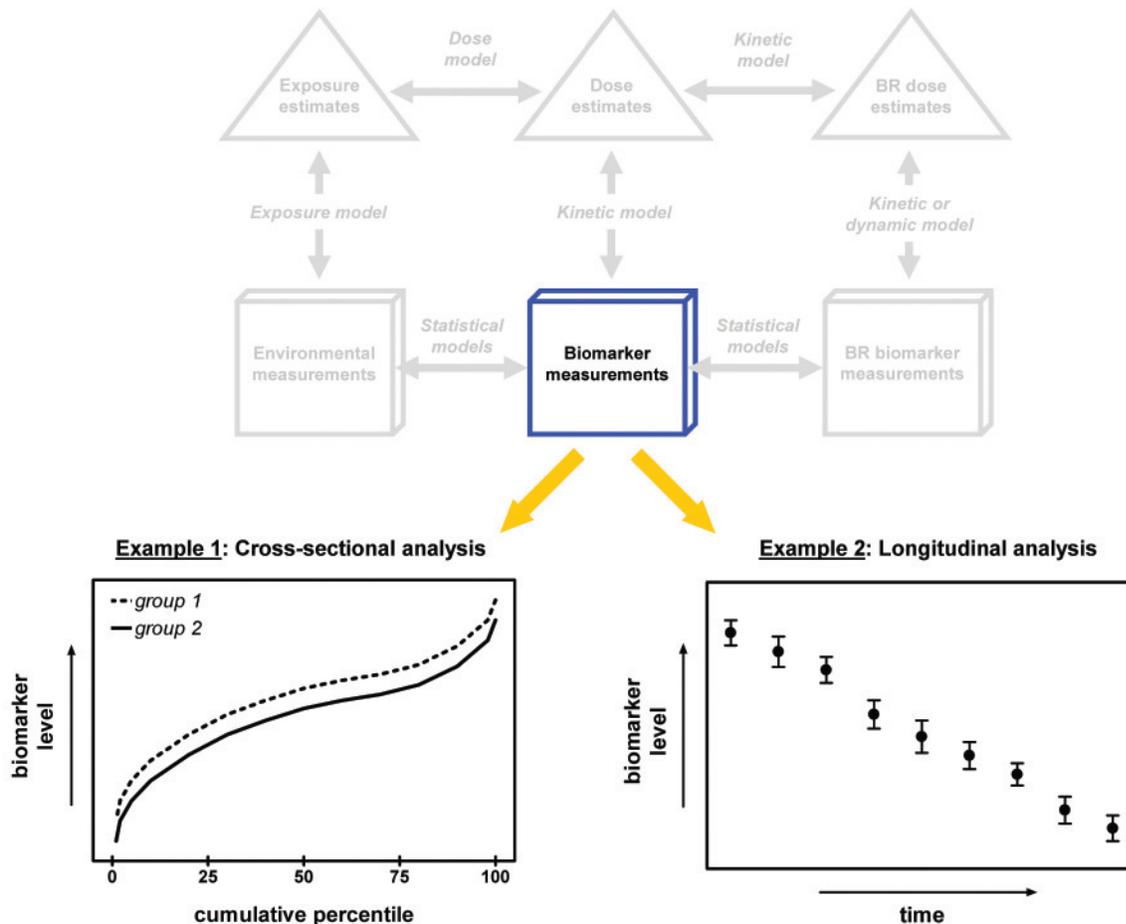


Figure 2. Requirements and examples of tier 1 analyses of biomarker data. (Gray objects are unavailable in a tier 1 analysis.)

(question 1 above) and increased risk of health impacts. In particular, these comparisons are used for evaluating exposures among vulnerable and susceptible subpopulations.

In example 2, longitudinal biomarker measurements for a population are shown decreasing over time, suggesting a similar decrease in exposure levels. Trends in longitudinal biomonitoring studies (either increasing or decreasing) can indicate a change in the source impacts on exposure (e.g., deregistration of a consumer product) or a change in human activities through which contact occurs (e.g., product use patterns). However, higher tier analyses of the biomarker data generally are needed to pinpoint the cause of a trend. As such, tier 1 longitudinal analyses of biomarker data are used to answer questions 2 and 3 above; that is, to identify chemicals with changing health risks from exposure and to prioritize chemicals for higher tier analyses.

3.2 Tier 2: Biomonitoring to support exposure assessment

Tier 2 analyses of biomarker data can answer the following questions to support exposure assessments.

1. What are the likely exposure sources?
2. What are the likely exposure routes?

As shown in Figure 3, tier 2 analyses consider environmental and biomarker measurements (paired at the subject level) and focus on statistical comparisons of these data. A graph in Figure 3 shows a regression of spot biomarker measurements on corresponding environmental measurements. A positive linear trend is shown in this example with a R^2 value of 0.3. This indicates that biomarker levels increased with increasing environmental levels, and that 30% of the biomarker measurement variance was explained by corresponding environmental measurements.

If, for example, the environmental measurements in this example were concentrations of a chemical in food, and the biomarker measurements were corresponding blood levels of the same chemical, then the results of the regression analysis would point to dietary ingestion as a likely exposure route. Furthermore, the results would point to food or, perhaps, a specific food item as an exposure source.

Considerable unexplained variance in the biomarker data (i.e., 70%), however, would suggest additional exposure routes and/or considerable covariate effects (e.g., timing of sampling events) on biomarker levels. Therefore, additional data would be necessary to better explain the observed biomarker variance and to further support the exposure assessment. These data could be part of a more complex tier 2 analysis (e.g., environmental measurements collected of different media to identify additional exposure routes) or of a hightier analysis as described in the next sections.

3.3 Tier 3: Biomonitoring to support risk assessment

Human health risk assessments for environmental chemicals traditionally are based on environmental measurements, observations of human activities, and information on chemical toxicity. Tier 3 analyses of biomarker data can be used to support risk assessments because they can answer the following questions:

1. What are the likely exposure levels?
2. What are the likely dose levels?

The requirements of a tier 3 analysis of biomarker data are shown in Figure 4 and build on the tier 2 parameters by adding exposure and dose models. Exposure and dose estimates are not linked directly to biomarker measurements in tier 3 analyses (see Figure 4) but can be indirectly linked via statistical models (e.g., multiple regression models) that collectively consider environmental measurements, human activities, and other covariate effects. Given these linkages, and that exposure and dose estimates can be compared to risk-based reference values (e.g., RfDs), tier 3 analyses can evaluate biomarker measurements within a risk context.

It is shown in Figure 4 that exposure and dose are estimated with environmental measurements, human activity data (included in exposure models), and uptake predictions but generally not with biomarker measurements. Biomarkers are, however, useful for evaluating exposure and dose estimates. If, for example, air levels of a chemical were not associated with corresponding biomarker levels, then exposure and dose estimates based on inhalation likely would be incorrect and, therefore, not comparable with reference values. On the other hand, strong associations between food and biomarker levels would support exposure and dose estimates based on dietary ingestion.

In our tier 2 regression example (Figure 3), we showed how measurements of a chemical in food explained 30% of the observed biomarker variance. This result suggests that exposure and dose estimates based on dietary ingestion would be reasonable, and therefore comparable to reference values for risk evaluation. However, given the added information in a tier 3 analysis, it would be possible to explain more biomarker variance, thus increasing confidence in the exposure and dose estimates.

For example, a graph in Figure 4 shows a regression of biomarker levels on covariate-adjusted environmental levels (as an analogue for dose). Here, the adjusted environmental levels reflect for each individual the combined effects of food concentration, food consumption, and factors affecting dietary uptake (e.g., food allergies). A regression R^2 value of 0.6 in this example suggests that the combined effects of food concentration and covariates could explain 30% more biomarker variance than food concentration alone (shown in Figure 3). Therefore, the additional information in tier 3 analyses can highlight other determinants of exposure e.g., activities and support exposure and dose estimates for risk assessment.

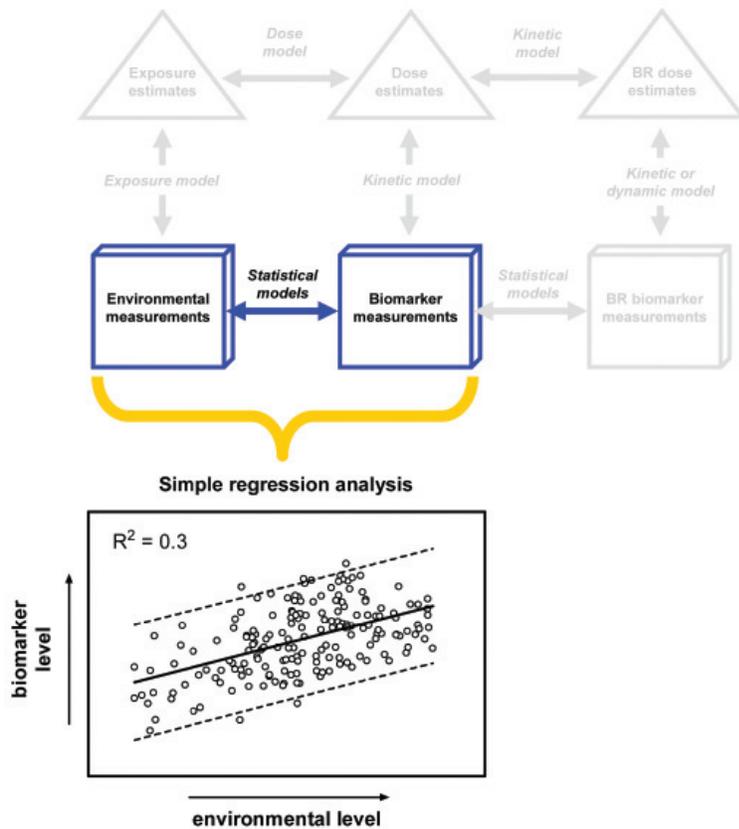


Figure 3. Requirements and an example of a tier 2 analysis of biomarker data. (Gray objects are unavailable in a tier 2 analysis.)

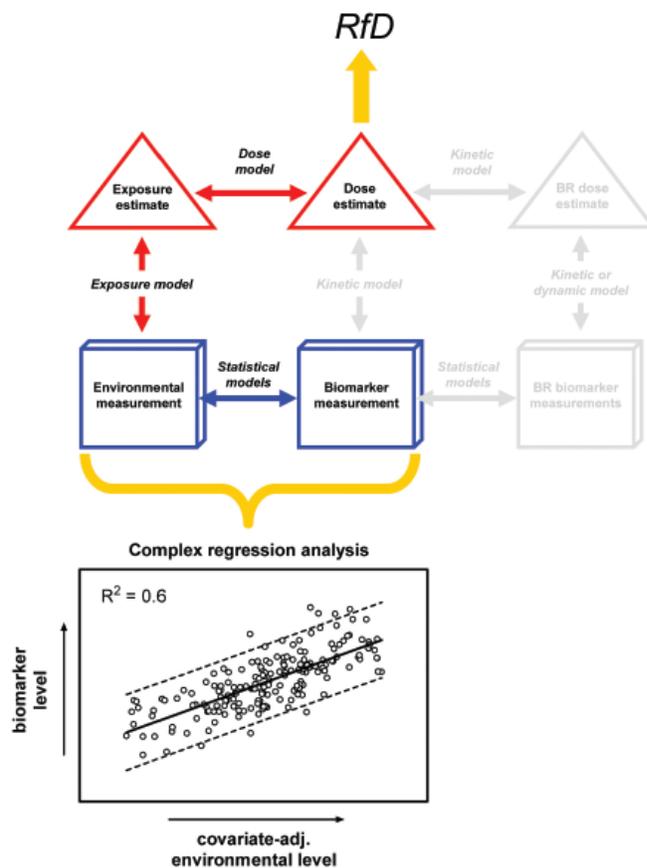


Figure 4. Requirements and an example of a tier 3 analysis of biomarker data. (Gray objects are unavailable in a tier 3 analysis.)

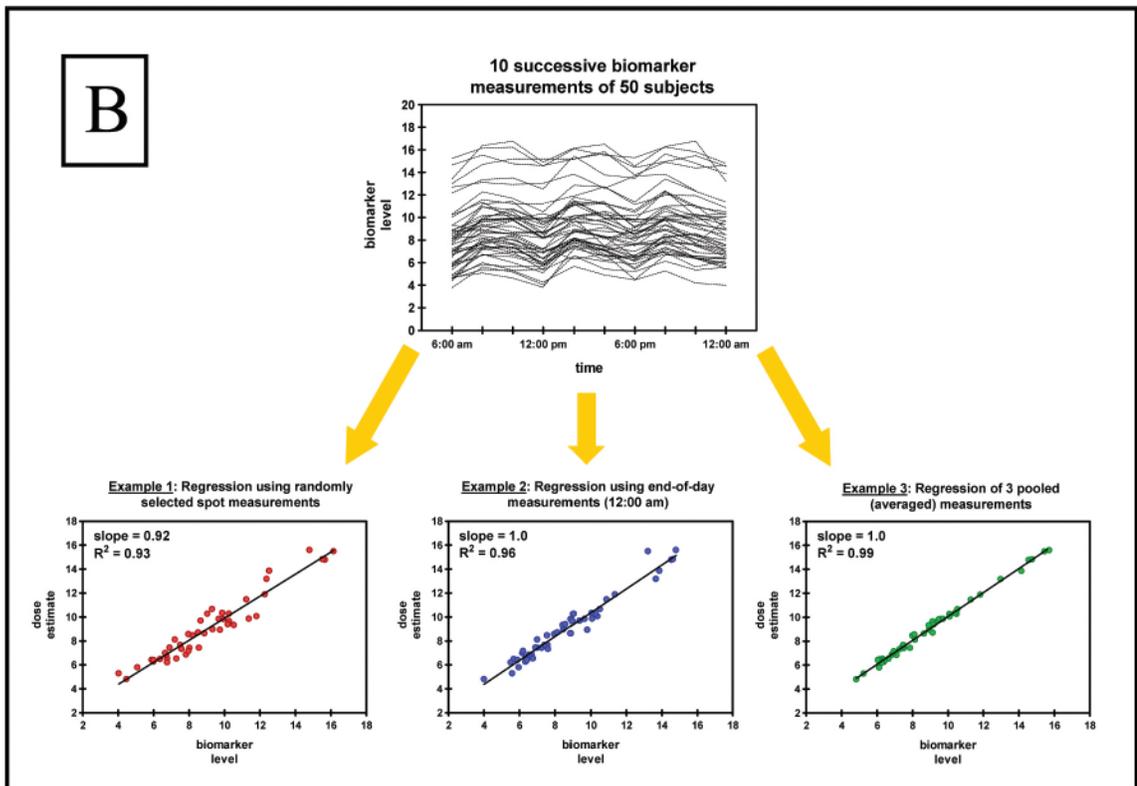
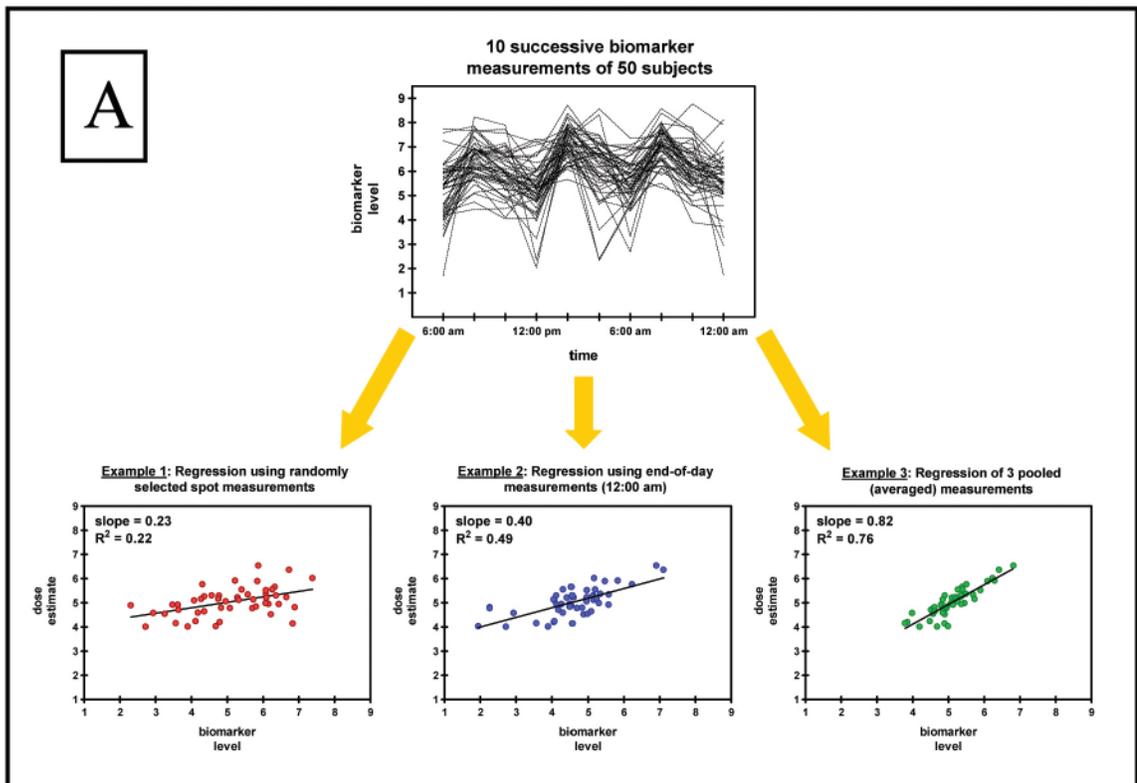


Figure 5. Requirements and examples of tier 4 analyses of biomarker data. (Gray objects are unavailable in a tier 4 analysis.)

3.4 Special consideration for timed events, sampling strategies, and repeated measures

Timed events (e.g., frequency and duration of exposure, time of biomarker sampling) for tier 2 and tier 3 analyses can impact the interpretation of biomarker measurements with respect to environmental measurements, exposure estimates, and dose estimates. The magnitude of these impacts largely depends on the between- and within-person components of biomarker variance.

Figures 5A and B show repeated biomarker measurements of individuals from two theoretical groups. Both figures show 10 consecutive measurements from 50 subjects, with the first measurements made at 6:00 a.m. and the final measurements at midnight (12:00 a.m.) on the same day. The biomarker levels in these figures vary between- and within-individuals according to dose levels and kinetic processes. In Figure 5A, biomarker measurements are highly varied over time for each individual and overlap considerably across individuals. These observations suggest that individuals have similar dose levels (based on daily average biomarker levels), and that chemical uptake and elimination occurs rapidly throughout the day. In Figure 5B, biomarker measurements are less varied with time, and are more easily distinguished between individuals. These observations suggest that individuals have different dose levels (long-term averages) and that kinetic processes occur more slowly.

Three example regressions of dose estimates on biomarker levels are given in both Figures 5A and 5B. Dose is approximated for each individual as their average biomarker level across all 10 measurements. In example 1 in both figures, dose is regressed on randomly selected spot biomarker levels; this simulates studies where one random biomarker measurement is made for each subject. Example 2 in both figures shows a regression of dose on end-of-day biomarker levels; this simulates studies where one biomarker measurement is made for each subject at a specific time point. In example 3 in both figures, dose is regressed on the average of three randomly selected measurements; this simulates studies where repeated measurements are made for each subject, and the measurements (or the biological samples themselves) are pooled (averaged) prior to analysis.

The regression results from the three examples in Fig 5B show very similar slopes (ranging from 0.92 to 1.0) and R^2 values (ranging from 0.93 to 0.99). These results indicate that timed events have little impact on biomarker interpretation with respect to dose when the between-person component variance is large. Specifically, these results suggest that the biomarker measurements from each of these examples could be used to accurately and precisely estimate dose levels.

In contrast, dissimilar regression results are shown from the three examples in Figure 5A, indicating increased impacts of timed events on biomarker interpretation when the within-person component of variance is large. The best linear association is shown in example 3 using the average of three random biomarker measurements (slope = 0.82, $R^2 = 0.76$). This suggests that repeated biomarker measurements are

preferred over spot measurements for improving the accuracy and precision of dose estimates. Furthermore, the regression slopes in Figure 5A show that spot biomarker measurements (collected randomly or at a fixed time) can severely underestimate dose levels when the within-person component of variance is large (attenuation bias).

3.5 Tier 4: Biomonitoring for exposure and risk assessments

Figure 6 shows that tier 4 analyses of biomarker data include the components for tier 3 analyses, as well as kinetic models to link dose estimates and biomarker measurements, and to predict BR dose levels. As such, tier 4 analyses can answer the following the questions for exposure and risk assessments.

1. What are the predominant exposure routes?
2. What are the best estimates of exposure and dose?
3. What is the estimated BR dose?

In the previous example of a tier 3 analysis (Figure 4), dose estimates and biomarker measurements were not directly linked. Rather, results from statistical comparisons were used as support for dose estimates. Risk-based decisions can be supported by statistical associations but can be further refined with an understanding of mass transfer from exposure to dose to biomarker levels; kinetic models are used to describe these mass transfer processes. More specifically, they are used to predict biological levels of chemicals and their metabolites following exposure events.

Example 1 in Figure 6 shows a theoretical comparison of observed and predicted biomarker levels over time. Here, the predicted values are estimated blood levels of a chemical following three dietary exposure events (e.g., breakfast, lunch, dinner). Assuming a well-parameterized and calibrated model, good agreement between predicted and observed values support the diet as the primary exposure route and help validate exposure and dose estimates. Alternatively, overestimation of the observed values would suggest incorrect exposure and dose estimates, whereas underestimation could suggest additional exposure routes or endogenous sources of the biomarker. In these situations, exposure and dose estimates could be reconstructed to be consistent with model predictions (discussion of exposure reconstruction is given in section 4.1.2).

Given the appropriate model parameters, the same kinetic models used to predict biomarker levels may be used to predict the BR dose. Example 2 in Figure 6 shows target levels over time of the same chemical from example 1. In this theoretical example, the parent chemical is neurotoxic, and the predicted values are brain tissue levels. As samples of brain tissue are generally unavailable in observational human studies, these predicted values are comparable only to observations from animals. Specifically, the area under the target-level curve (AUC_{target}, which is the time-integrated BR dose) or the maximum level at the target, could be interpreted

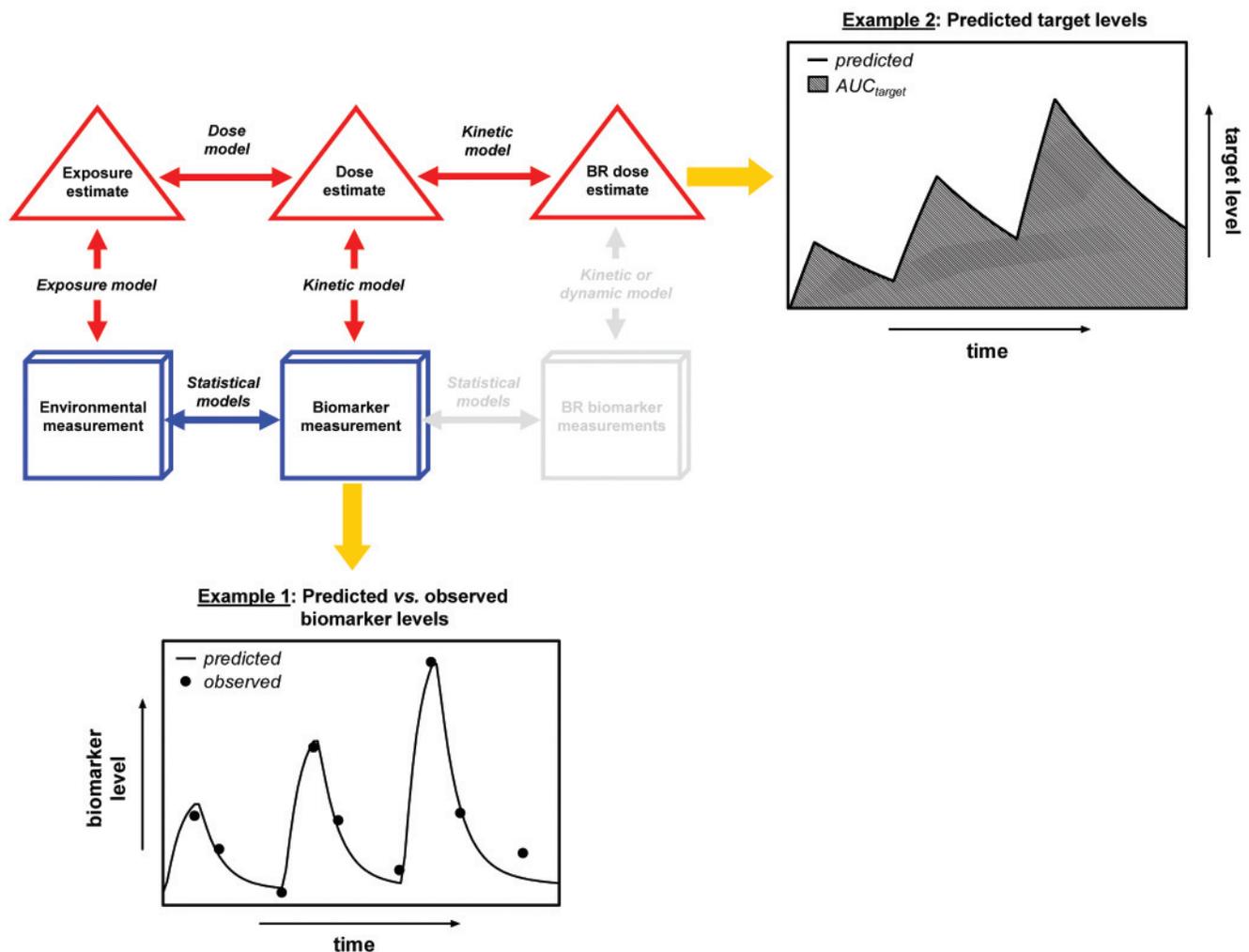


Figure 6. Requirements and examples of tier 4 analyses of biomarker data. (Gray objects are unavailable in a tier 4 analysis.)

based on existing dose-response relationships. These types of comparisons can inform exposure impacts on health risks but may be refutable because of missing biomarker measurements at the target (i.e., BR biomarker measurements).

3.6 Tier 5: Biomonitoring to advance exposure and risk assessments

Tier 5 analyses of biomarker data include all components of the source-to-outcome continuum, as shown in Figure 7. That is, tier 5 analyses predict biomarker and BR biomarker levels for comparison to measured values. These comparisons enable tier 5 analyses to answer the following research questions to advance exposure and risk assessments.

1. What are the best estimates of BR dose?
2. What are the likely impacts of exposure on health outcome?
3. What other factors may affect health outcome?

The previous section demonstrated how, in tier 4 analyses, BR dose can be estimated and interpreted using existing dose-response relationships. Because the BR dose estimates in tier 4 analyses are not confirmed with measured values,

there is uncertainty in model predictions. Tier 5 analyses can reduce this uncertainty via comparison of predicted and observed BR biomarker levels.

Example 1 in Figure 7 shows predicted versus observed levels of a BR biomarker. This is an extension of the examples in Figure 6 where the brain was a target tissue, and the stressor was a chemical neurotoxin found in food. In this example, enzyme inhibition (e.g., cholinesterase) in the brain was the desired outcome, but, because brain tissue is generally inaccessible, blood enzyme levels were used as surrogates. Kinetic and dynamic models were used to predict blood enzyme levels following three theoretical dietary exposure events. Predicted and observed levels were then compared to evaluate the BR dose estimate.

In example 1, good agreement between measured and predicted values indicates an accurate estimation of BR dose and a good understanding of dynamic processes. Therefore, the biomarker measurements in this example could be quantitatively linked backward to exposure (dietary intake) and forward to potential health outcome (effects caused by inhibited enzyme levels). However, poor agreement between BR biomarker levels (observed versus predicted), combined with accurate dose estimates from tier 4 analyses, would suggest

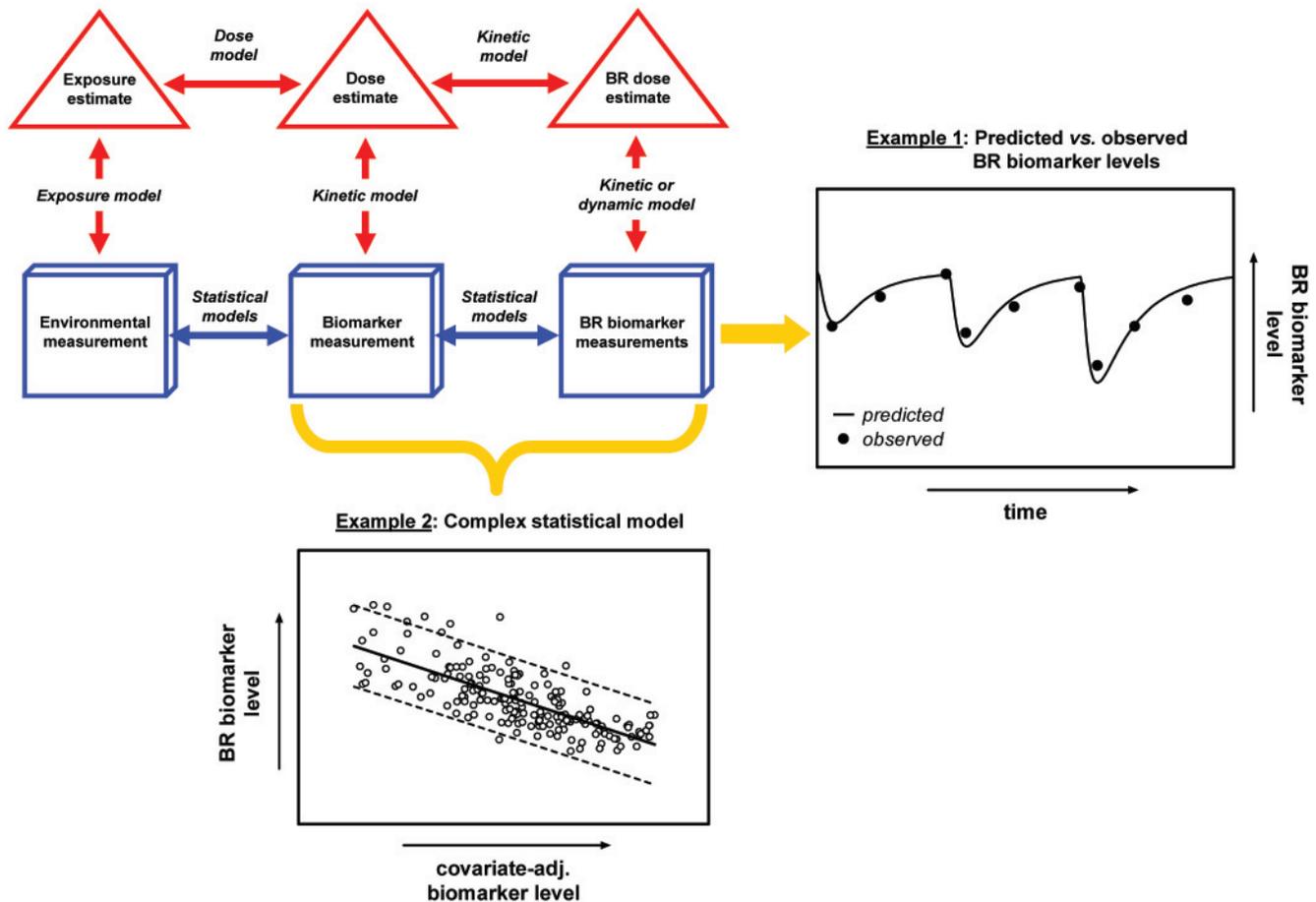


Figure 7. Requirements and examples of tier 5 analyses of biomarker data. (Gray objects are unavailable in a tier 5 analysis.)

an incomplete understanding of dynamic processes *in vitro* (possibly caused by uncertainties in interspecies extrapolation). For example, overestimation of BR biomarker levels could suggest the omission of important recovery processes, whereas underestimation could suggest additional exogenous or endogenous sources. In these instances, clarification would be necessary before utilizing biomarker measurements from observational studies for health effects research.

In addition to kinetic and dynamic models, statistical comparisons of biomarker and BR biomarker measurements are used in tier 5 analyses to elucidate exposure impacts on health outcome. These comparisons are generally more complex than those in lower tier analyses, given repeated observations of individuals. For example, *in vitro* dose-response associations can be informed using regressions of BR biomarker levels (representing response) on biomarker levels (representing dose). Nondose related effects also can be observed by including in the models covariates such as age, gender, family health history, and genetic information.

Example 2 in Figure 7 shows a regression of BR biomarker levels on covariate-adjusted biomarker levels. Continuing from the previous example, this plot suggests that blood enzyme levels decreased with increasing adjusted biomarker

levels. In other words, normal biological activities were suppressed given elevated dose levels. The effects of individual predictor variables on BR biomarker levels could be examined to further explain this observation. Moreover, the model results could help identify important *in vivo* processes that could improve dynamic models. Overall, the combined results of the kinetic, dynamic, and statistical models could inform exposure *and* susceptibility effects on health outcome.

This section presented a simple biomonitoring framework aimed at improving biomarker use and interpretation in exposure and health research. Throughout the section, simple definitions and examples were given to articulate uses of biomonitoring data. These examples were not meant to represent all biomonitoring research options but, rather, to serve as a road map for establishing new studies and for interpreting existing biomarker data. A summary of the uses and requirements of the five biomonitoring tiers is given in Table 1. Table 1 lists specific questions that can be answered in a tiered analysis, as well as the measurements, models, and model estimates that are required to complete an analysis.

Table 1. The uses and requirements of the five biomonitoring tiers.

Tier	Primary Uses	Measurements Needed	Models Needed	Estimated Values
1 ^a	Exposure surveillance: <i>Who is exposed?</i> <i>What are the exposure trends?</i> <i>Which chemicals should be prioritized for higher tier analyses?</i>	1) Biomarker	<i>None</i>	<i>None</i>
2 ^a	Supporting exposure assessment: <i>What are the likely exposure sources?</i> <i>What are the likely exposure routes?</i>	1) Environmental 2) Biomarker	1) Statistical	<i>None</i>
3 ^a	Supporting risk assessment: <i>What are the likely exposure levels?</i> <i>What are the likely dose levels?</i>	1) Environmental 2) Biomarker	1) Statistical 2) Exposure 3) Dose	1) Exposure 2) Dose
4 ^b	Exposure and risk assessment: <i>What are the predominant exposure routes?</i> <i>What are the best estimates of exposure and dose?</i> <i>What are the likely BR dose levels?</i>	1) Environmental 2) Biomarker	1) Statistical 2) Exposure 3) Dose 4) Kinetic	1) Exposure 2) Dose 3) BR dose
5 ^b	Advancing exposure and risk assessment: <i>What are the best estimates of BR dose?</i> <i>What are the likely impacts of exposure on health outcome?</i> <i>What other factors may affect health outcome?</i>	1) Environmental 2) Biomarker 3) BR biomarker	1) Statistical 2) Exposure 3) Dose 4) Kinetic 5) Dynamic	1) Exposure 2) Dose 3) BR dose

^a Single or repeated measurements

^b Sepeated measurements only

4.0 Filling in Information Gaps

As described in the previous section, the ability to utilize biomonitoring data to assess human exposures and to evaluate human health risks depends on the availability of various types of information. Besides collecting additional exposure, kinetic, or toxicity data, there are other approaches that can be used to inform missing elements in the source-to-outcome continuum, thus enabling biomonitoring data to be more fully utilized in exposure and risk assessments. Approaches presented in this section include exposure and dose modeling, *in silico* modeling, and chemical surrogates, as well as the exploration of ‘omics biomarkers.

4.1 When data informing exposure estimates are missing

Problem: When data that are used to inform exposure estimates are insufficient (e.g., missing environmental measurements), it is difficult to identify exposure pathways and routes based on biomarker measurements. In this case, biomarker measurements may only be useful as a surveillance tool (i.e., tier 1 approach) or for developing hypotheses for future research.

Potential solutions: In quantitative exposure assessment, human exposures are estimated by combining environmental measurements with information regarding human activities, demographic and activity attributes of the intake/uptake rates, and other exposure factors. Given corresponding measurement and activity data, simple formulas (e.g., concentration \times contact time \times exposure factors) with point estimate inputs can be used to estimate exposure. In the absence of corresponding exposure information, advanced probabilistic simulations and mathematical algorithms can be used to generate exposure and intake dose estimates. NERL’s Stochastic Human Exposure and Dose Simulation (SHEDS)⁴ is an example of such an advanced model. The output of these probabilistic exposure models can be used as input terms into pharmacokinetic models to further describe the exposure- and dose-biomarker relationship.

Limitations: Besides the technical quality of the model development process, the predictive ability of an exposure model depends largely on the representativeness, relevancy, and quality of the input data. When possible, input data should be obtained through carefully designed observational exposure or survey studies (e.g., the Food and Drug Administration’s Total Diet Survey) that are representative of a given population of interest. An exposure model that is built

based on these input data, however, is generally unsuitable for predicting biomarker levels at the subject level because of using population and non subject-specific inputs. Instead, it is more appropriate to use probabilistic models to generate a distribution of estimated biomarker concentrations to compare with an observed distribution of biomarker concentrations. These distributional comparisons may be helpful for identifying potential exposure sources, pathways, and routes.

4.2 When kinetic information is missing

Problem: Kinetic data inform the specific metabolism/biotransformation pathways of xenobiotic chemicals within a biological system. Without this kinetic information, parameterization and performance of a pharmacokinetic model, which is a predictive tool for describing the time course of the exposure-biomarker relationship, become highly uncertain.

Potential solutions: In the absence of kinetic data, chemoinformatics-based techniques, such as quantitative structure-activity relationship (QSAR), can provide pragmatic estimations of chemical-specific parameters for a provisional pharmacokinetic model. Currently, many software packages (e.g., MOE⁵ or QikProP⁶) exist whereby one can develop, augment, and utilize new or existing QSAR. In addition, there are also “trainable” QSAR models, such as ACD/Labs’ “*Suite of predictors for PhysChen, ADME, and Tox,*” that may be used to adapt and filter a model’s predictive capability based on chemical similarity indices.

Limitations: The effective use of QSAR or any molecular model relies on the understanding of a model’s domain of applicability. When developing a QSAR model, a specific set of chemicals (training set) is used to parameterize the model. Bounded by the molecular properties of the training set, a QSAR model is limited to a specific chemical space. In other words, a QSAR model is best suited for interpolating data within the model specification, but ill-suited for extrapolating outside the chemical space of the training set. To evaluate chemicals outside of this chemical space, one will need to reparameterize an existing model or to create a new one. In such cases, the best approach is to generate relevant *in vitro* chemical data to inform QSAR modeling efforts.

⁴ http://www.epa.gov/heasd/products/sheds_multimedia/sheds_mm.html

⁵ MOE (Molecular Operating Environment) is a software package developed by Chemical Computing Group, Inc. It contains Structure-Based Design, Pharmacophore Discovery, Protein & Antibody Modeling; Molecular Modeling & Simulations, Cheminformatics & HTS QSAR, and Medicinal Chemistry Applications.

⁶ <https://www.schrodinger.com/products/14/17/>

4.3 When toxicity information is missing

Problem: Toxicity information provides an anchor for a chemical in a risk context. Although having toxicity data would not necessarily be sufficient to evaluate risk, in lieu of data on apical toxicity, no clear risk assessment or risk management activity can be performed. Thus, alternative methods for estimating non-existent toxicity information are discussed here.

Potential solutions: In addition to being used to parameterize pharmacokinetic models, chemical surrogates and *in silico* models may be used to infer toxicity when no such data exist. Chemical surrogates provide a starting point for interpolating toxicity between chemicals. For instance, nonane data may be an adequate surrogate for decane data if there is an established trend of toxicity or other relevant data with alkane chain length (i.e., in the case of nonane with C6, 7, 8, and 10 alkanes). When chemical surrogates are unavailable or deemed unreliable (e.g., low chemical similarity), the relationship of chemical structure to toxicity may be estimated using *in silico* predictions. Several *in silico* techniques exist by which predictions are made using chemical functional group approaches. In this approach, the potential for health risk is determined by a chemical substructure search against a precomputed set of structural fragments that give rise to toxicity alerts (e.g., the Osiris Property Explorer, <http://www.organic-chemistry.org/prog/peo/>, and ADME/Tox Boxes, <http://pharma-algorithms.com/webboxes/>). Aside from estimating potential for health risks, QSARs can also be generated in the same manner to quantify toxicity on the basis of chemical descriptors – historically, this has been reported for biological outcomes such as Lethal Dose, 50% (LD50) in rats and mice.

Limitations: The use of chemical surrogates relies heavily on the following two assumptions: (1) the mode of action is preserved across a class of chemicals, and (2) chemical similarity among chemical class constituents is highly conserved. Experimental validation of actual toxicity still should be carried out when possible, and care should be exercised when choosing index chemicals in the context of chemical similarity. For both chemical surrogates and *in silico* approaches, false positives and false negatives may reside in the predicted outcomes because of inherent model structure assumptions and/or training set limitations. In addition, to develop a reliable *in silico* approach, relevant toxicity data and relationships must exist.

4.4 When biologically relevant biomarkers are unidentified

Problem: To date, few biomonitoring studies have incorporated both biomarkers and biologically relevant biomarkers to expand the traditional biomonitoring study through the further mapping of biologically relevant biomarkers to toxicity starting points. This paucity in the combined use of biomarkers and biologically relevant biomarkers stems, in part, from insufficient routine analytical methods with appropriate quality assurance documentation for known biomarkers of interest.

Potential solutions: Researchers have incorporated new cost-effective, high-sample-capacity analytical methods into biomonitoring studies to help quantify chemical levels in environmental and biological samples and to determine new ‘omics-based biomarkers and BR biomarkers with which to link exposure sources to health outcomes. A more detailed discussion of such ‘omics-based biomarker research is provided below.

Proteomics is a bioanalytical tool that identifies proteins that are altered through interactions with environmental chemicals. The concept of protein expression signatures is based on the measureable protein responses to chemicals in animal studies. Sensitive, precise, and fast multianalyte methods for measuring proteins in parallel can lead to protein fingerprinting to aid in identifying new biomarkers and BR biomarkers. In addition, multiplexed immunoassays (e.g., microarrays) are becoming robust and reliable tools for high throughput proteomic analyses to study the structure and functional interactions between proteins and how these interactions control complex processes in biological systems.

Another emerging technology that shows promise in biomonitoring research is metabolomics. Metabolomics includes the study of toxicant-induced perturbations in endogenous metabolites that result from exposures. Metabolomic studies conducted on various species have shown that systematic patterns of change occur in the metabolome following exposures to pesticides and other environmental chemicals. The integration of metabolomics with proteomics and genomics has great potential for providing the systems biology information that will elucidate the complex relationships between measurements of biomarkers/BR biomarkers and health effects.

It is important to note that, in the context of the ‘omics technologies, a biomarker is a unique pattern of a large number and variety of endogenous gene/protein/metabolite/biochemical changes. It has been proposed that these signatures (or fingerprints) may be more informative and more chemical-specific or exposure-pathway-specific than one or a few conventional biomarkers (chemical concentrations in blood, tissue, or excreta). Also, the flux (or dynamic time course) of changes in ‘omics pattern may yield a better estimate of the time of occurrence of a single-event exposure. Furthermore, ‘omics pattern changes may persist well after the chemical stressor has cleared the body, which will be important when interpreting biomonitoring results for nonpersistent chemicals. Finally, because ‘omic pattern changes often can be linked to a specific mechanism of action, they may enable identification of the harmful component following exposure to a chemical mixture. Although these concepts have not yet been fully tested and proven, preliminary work suggests that studies with conventional biomarkers could be improved significantly by augmenting them with information from ‘omic techniques.

Preliminary work in NERL suggests that Nuclear Magnetic Resonance (NMR)-based metabolomics may be particularly appealing in biomonitoring studies, in large part because the technique is well suited for relatively high-throughput analysis. For example, the per-sample cost is low, little sample preparation is required, and the instrument can be configured for automated analysis. This is important when designing experiments to establish endogenous biomarkers as indicators of exposure because these investigations require analysis of a

great many samples (e.g., as a function of contaminant identity, magnitude, duration and timing of exposure). In addition, the technique is readily amenable to blood as well as biofluids that can be taken noninvasively from humans (e.g., urine, saliva, breath condensate).

Limitations: All of these highly multiplexed approaches require well-characterized methods that are cost-effective. Reagents must be standardized and screened in a multiplexed environment before use. Methods development must encompass quality assurance measures, standard operating procedures, data handling, interpretation, and reporting. Current informatic systems are pressed to keep pace with the increasing multiplexing capability of microarrays and flow cytometry methods as the interpretation of data is extremely challenging. Finally, linking ‘omics-based biomarkers’ or biologically relevant biomarkers to exposures or health outcomes requires significant resources.

5.0 Additional Considerations for Interpreting Biomonitoring Data

Even when sufficient measurement data and predictive models exist to fill in each component of the source-to-outcome continuum (Figure 1), there are still many uncertainties and data gaps that can complicate the interpretation of biomonitoring data. In this section, some of these complications are discussed and approaches to minimize their impact on the interpretability of biomonitoring data are recommended.

5.1 Categories and uses of biomarkers⁷

The study design and interpretive options for a biomonitoring study depend largely on the category to which a biomarker belongs, as well as on the previously available information. As such, there are sometimes surprises lurking in otherwise simple biomonitoring strategies when a researcher misclassifies a biomarker. Thus, it is important to be aware of the category to which a biomarker belongs, as well as the potential inferences and uses in each category.

Biomarkers can be partitioned into four basic categories based on their origin.

Group 1. Exogenous (native) chemicals are comprised of exogenous biomarkers that are anthropogenic in origin and are not formed via human oxygen/hydrocarbon metabolism. Examples of exogenous chemicals include dioxins, polycyclic aromatic hydrocarbons (PAH), benzene/toluene/ethylbenzene/xylenes (BTEX), polychlorinated biphenyls (PCB), and organophosphate (OP) pesticides. Because these chemicals have no endogenous sources, they can usually be attributed to their respective environmental sources. It may be noted that there may be multiple sources and routes of exposures and, therefore, deducing the specific pathway requires additional metadata, such as personal activity, geographic location, occupation, and environmental measurements.

Group 2. Endogenous metabolites are comprised of a great variety of “life process” chemicals widely studied in the medical community, and they may be mapped quantitatively to a toxicological initiating event. Examples of endogenous biomarkers include cholesterol, liver enzymes, fatty acid

esters, urea, triglycerides, cytokines, and creatinine. These biomarkers generally have no major environmental sources. They, however, can be used to provide a baseline or a statistical range for “normality” within a non- or minimally exposed subpopulation; as such, outlier individuals can be spotted quickly and further evaluated to determine whether there is an environmental chemical that triggers those biological responses.

Group 3. Ubiquitous organic compounds contain a wide variety of chemicals that are known, trace-level, human metabolites that are also present in similar concentrations in the environment. Examples of ubiquitous organic compounds include ketones, aldehydes, alcohols, phenols, amines, and organic acids. Mostly, these compounds pose a dilemma in microenvironments where, for example, an endogenous exhaled chemical from subject A becomes an exogenous inhalation exposure for subject B. As long as confounding sources are properly identified and monitored, this group of chemicals may aid in predicting health outcome or assessing current health status when case-control or pattern recognition strategies are used.

Group 4. Phase-1 and Phase-2 metabolites are exogenous biomarkers but differ from Group 1 in that the biomarkers are metabolites formed by biological processes known as phase-1 and phase-2 metabolism. Group 4 biomarkers are the most frequently used biomarkers in exposure assessment. Phase-1 and -2 metabolism generally results in formation of more polar species that can be eliminated readily eliminated in urine or feces. Phase-1 metabolism involves oxidation, reduction, or hydrolysis of the parent chemical. Examples of phase-1 metabolites include 3,5,6-trichloro-2-pyridinol (a metabolite of chlorpyrifos) and mono-ethyl phthalate (a metabolite of phthalate). Phase-2 metabolites are the result of further conjugation reactions between phase-1 metabolites and larger biomolecules such as glutathione, glucuronic acid, sulfates, and other peptides. Some Phase-1 metabolites or their conjugated forms are electrophilic and form stable adducts with nucleophilic sites on proteins and DNA. Phase-2 metabolites are measured primarily in blood and urine, although analytical methods also exist for other media, such as exhaled breath condensate and bronchoalveolar lavage fluid.

⁷ The four categories and uses of biomarkers are published in Tan et al. (2012). Reconstructing Human Exposures Using Biomarkers and Other “Clues”, *Journal of Toxicology and Environmental Health, Part B*, 15(1), 22-38.

5.2 Different categories of chemicals based on their biological half life in relation to exposure patterns

Information regarding the half-life of a chemical in the sampled tissue can be used to determine the exposure period reflected by a biomarker measurement. If a chemical has long half-life, the biomonitoring data are likely to reflect long-term exposures. If a chemical has short half-life, the biomonitoring data often reflect the daily variation in exposure patterns. Based on a priori knowledge regarding a chemical's biological persistence and exposure patterns, NRC (2006)⁸ categorizes chemicals into one of four groups: (1) lipid-soluble, bioaccumulative chemicals at steady state exposure; (2) lipid-soluble, bioaccumulative chemicals not at steady state exposure; (3) shorter half-life chemicals at pseudosteady state exposure; and (4) short half-life chemicals that do not approach steady state. For each category, NRC has provided an example to demonstrate how human pharmacokinetic models can be used to convert biomonitoring data to exposure dose when kinetic and exposure data are available.

Unfortunately, kinetic or exposure data do not exist for a majority of chemicals. When persistence data are lacking for a chemical, QSAR can be used to infer persistence on the basis of structural similarity to chemicals of known persistence. In the absence of exposure data, NERL has the expertise to collect data on exposure pathways and environmental concentrations. Given that exposure and persistence information is obtainable, different modeling approaches can be applied to estimate exposure/dose based on biomarker measurements.

- For persistent chemicals that have half-lives in the order of months to years, their long half-lives tend to smooth out daily variations in exposure. In other words, a biomarker measurement is more likely to reflect chronic exposures or a single historical exposure. For these chemicals, a simple pharmacokinetic model with first-order clearance can be used to estimate exposure dose for a given biomarker measurement. For lipophilic chemicals, life-stage simulation will need to be considered.
- For semi-persistent chemicals that have half-lives in the order of days to weeks, their biomarker concentrations reflect exposures over a period on the order of a few half-lives prior to sampling. For these chemicals, it is critical to identify the time of biomarker collection with respect to exposure events (e.g., the last meal eaten). If this information is obtainable from biomonitoring studies, exposure-pharmacokinetic modeling can be conducted to identify key exposure events reflected by a biomarker measurement. Otherwise, exposure pharmacokinetic modeling can be used only to identify the set of exposure scenarios plausibly associated with the biomarker measurements.

⁸ NRC (National Research Council) (2006). Human Biomonitoring for Environmental Chemicals. National Research Council Committee on Human Biomonitoring for Environmental Toxicants. National Academies Press, Washington, DC.

- For nonpersistent chemicals that have half-lives in the order of minutes to hours, their biomarker concentration is heavily influenced by recent exposure events. Uncertainties in time and duration of transient exposures make interpreting these biomarker results challenging. One approach for dealing with nonpersistent chemicals for which limited exposure information exists is to use a probabilistic approach that estimates a distribution of exposures that is consistent with the observed distribution of biomarker measurements.

5.3 Exposure reconstruction⁹

Although most biomonitoring surveys only have data for a tier 1 analysis, the call for exposure reconstruction from biomarker data has challenged the use of all available biomonitoring data to reconstruct exposures. This call is motivated by the traditional risk assessment paradigm, and the resulting estimates of safe “exposures” are based on measures of administered dose (e.g., RfD, NOAEL, LOAEL) or environmental concentrations (e.g., reference concentration [RfC], maximum contaminant level [MCL], National Ambient Air Quality Standards [NAAQS]). In cases where exposure guidance values exist, dose levels or environmental concentrations converted by biomarker measurements can then be compared with exposure guidance values for assessing human health risks (Figure 8).

NRC (2006) recommended two main computational approaches for such conversion.

- a. Use human pharmacokinetic models to convert biomarker measurements to dose levels that are comparable to an RfD or other dose-based toxicity value (reverse dosimetry)
- b. Use animal pharmacokinetic models to convert the administered dose-response relationship from toxicology studies to a target site dose-response relationship that can be used to evaluate human biomonitoring results (forward dosimetry)

Both approaches begin with either a well-validated kinetic model (or physiologically based pharmacokinetic (PBPK) model, often originating from controlled animal studies) or a provisional model that is structurally representative and complete as possible based on available kinetic data. Ideally, if a human model exists or can be constructed using time course data from controlled human studies, and the biomarker of interest is one of the model outputs, then the availability of exposure information will determine whether a biomarker measurement can be used to reconstruct exposure concentrations. In cases where reliable exposure information exists, a human pharmacokinetic model can be utilized to reconstruct exposure concentration as the only unknown exposure parameter (Figure 9, Steps 1, 2, and 3). The caveat is that some variability and uncertainty in pharmacokinetics still exist around the estimated exposure concentrations. When

⁹ Part of this subsection is published in Tan et al. (2012). Reconstructing Human Exposures Using Biomarkers and Other “Clues”, *Journal of Toxicology and Environmental Health*, Part B, 15(1), 22-38.

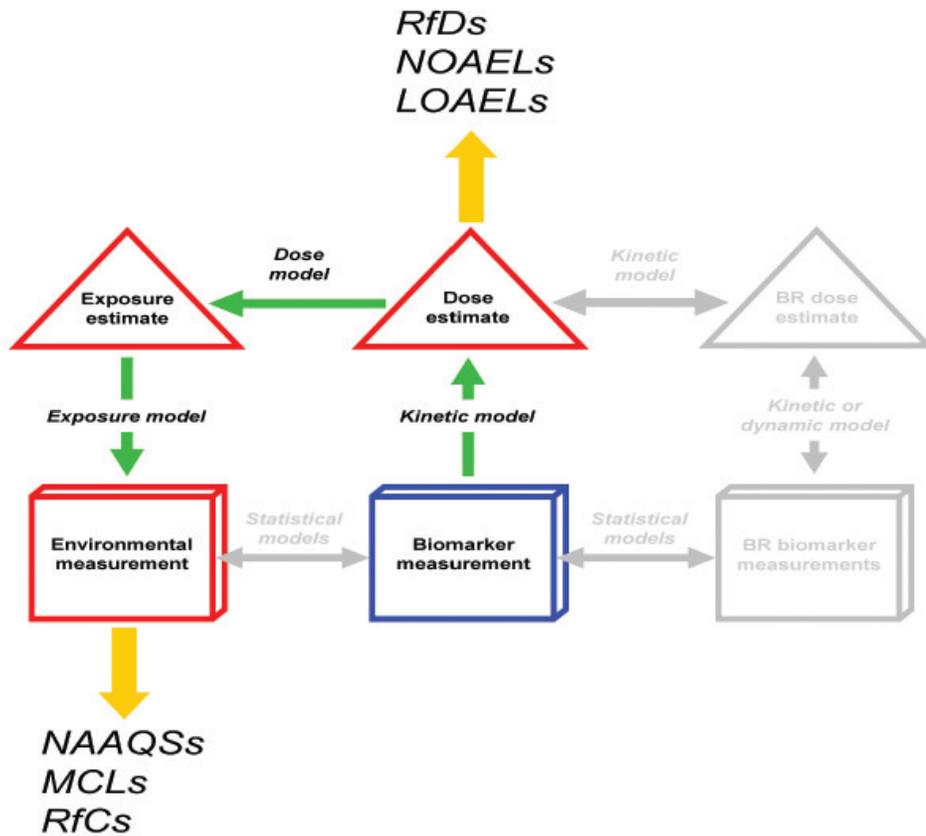


Figure 8. Biomarker measurements to estimate dose levels, exposure levels and environmental stressor levels for comparison to reference values. The blue box shows measured values, the red triangles and box show estimated values, and the green arrows show the reconstruction pathways.

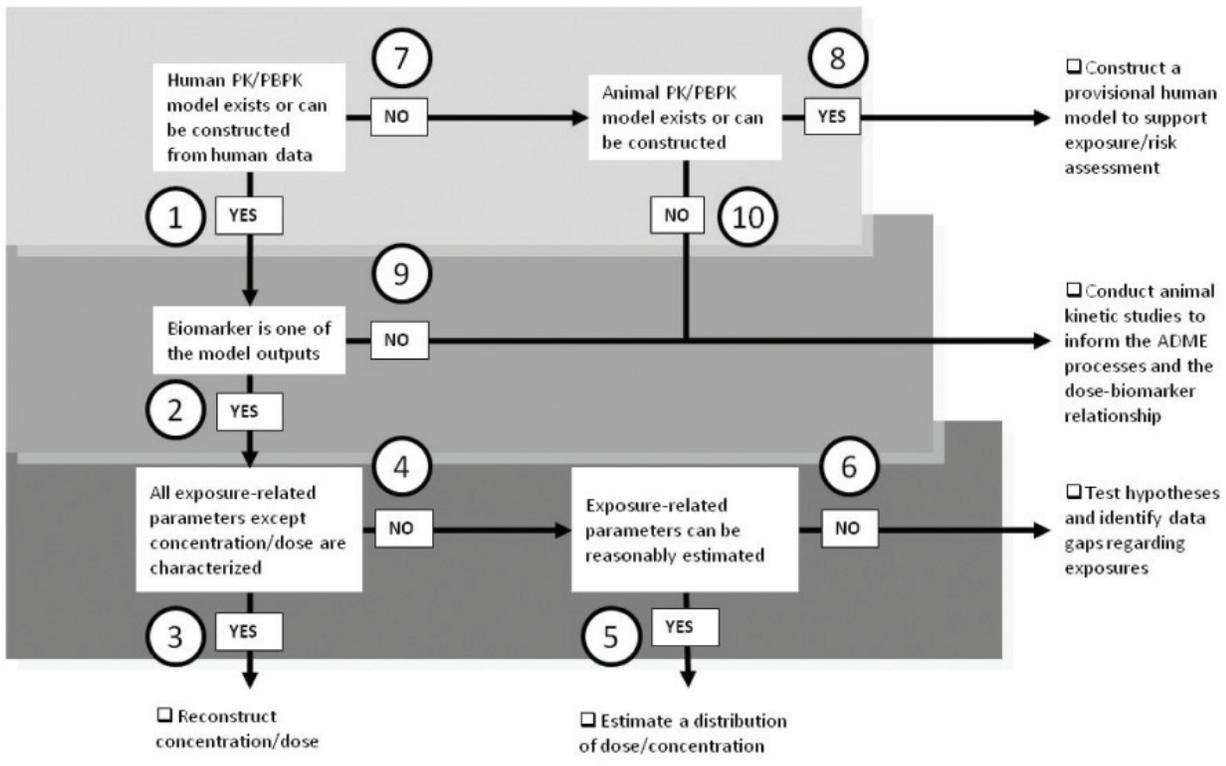


Figure 9. The type of exposure information that a biomarker measurement can infer depends on the availability of kinetic and exposure data.

large variability and uncertainty are expected for other exposure parameters (e.g., duration, frequency), a probabilistic approach should be conducted for estimating a distribution of potential exposure concentrations (Figure 9, Steps 1, 2, 4, and 5). In the case where exposure pathways are not well characterized or understood, a pharmacokinetic model only can be used to test hypotheses of potential exposure scenarios and identify data gaps regarding exposure information (Figure 9, Steps 1, 2, 4, and 6).

If most exposure information can be obtained, different modeling approaches can be applied in exposure reconstruction based on the biological half-life of the chemical in body (see Section 5.2). When only an animal pharmacokinetic model is available, a provisional human model may be constructed to identify exposures of greater concern or to complement other exposure measurements to assist in exposure and risk assessment (Figure 9, Steps 7 and 8).

A provisional model is expected to produce a “first approximation” of chemical disposition that requires additional human data to support. Sometimes, when the biomarker of interest is not one of the model outputs (Figure 9, Steps 1 & 9), or neither a pharmacokinetic model nor kinetic data exist (Figure 9, Steps 1, 7, and 10), animal kinetics studies will need to be conducted to inform the ADME process and the dose-biomarker relationship. These *in vivo* data can be used to develop and parameterize an animal pharmacokinetic model, which can subsequently be “scaled up” to a provisional human model. Alternatively, *in silico* molecular models may be utilized to parameterize a provisional human model. The provisional model then can be used to test hypothesis or to identify data gaps.

5.4 Nondetect data

Analytical chemistry methods have limits of detection (LOD) and limits of quantification (LOQ) that rarely (if ever) include zero. Improvements in analytical capability would be expected to lower the LOD and possibly the LOQ toward zero, but never at zero. In addition, it is problematic to treat nondetects statistically.¹⁰ Assigning zero to nondetects is a problem for transformation (angular, square root, or logarithmic) to approximate the normal distribution. A large number of zero values is equally challenging for normal score transformation (too many ties) and the use of distribution-free procedures. One common solution to this problem is to truncate the distribution of environmental/biomarker measurements at the LOD and to consider only the positive values for statistical analysis. This approach, however, would suggest that the nondetect values are uninformative or missing values rather than true values indicative of low or nonexposure. Censoring zero values when they are true is like imposing a type 1 error, rejecting a hypothesis that is actually true, before the start of the test. In the case of biomonitoring in which comparisons are sought between populations, subpopulation, and cohorts by time and location, left-truncation leaves the Poisson or log-normal distributions biased in favor of positive (overestimated) exposure.

An alternative to left-truncation of a continuous variable involves imputation of replacement values for non-detects (allowing some to be actual zero) at the relevant LOD (e.g., $2/3X$, $X/2$, $X/\sqrt{2}$). However, this imputation assumes that the nondetect values are members of that same population (i.e., each trial has only a single possible outcome). Thus, a test of normality is expected to follow. Rejection of the hypothesis of normality would render the need for the imputations moot, and consideration must be given to alternative binomial or multinomial treatments (i.e., each outcome can have two or more possible outcomes).

5.5 Nonspecific biomarkers

The presence of nonspecific biomarkers is relatively common. Nonspecific biomarkers can arise when multiple parent chemicals can yield the same metabolite that is the biomarker or when both the parent chemical and its degradate (which is also the metabolite used as the biomarker) co-occur in the environment. Concurrent exposures to multiple chemicals that yield the same biomarker can lead to false positive interpretations related to an exposure. Successful attribution of nonspecific biomarkers to actual exposure lies with studious understanding of metabolism and pharmacokinetics, combined with knowledge of the spatial and temporal conditions of exposures. Additional information of environmental conditions, such as prior determination of ratios of chemical of interest/other chemicals can ameliorate false-positive interpretations and make dose-biomarker relationships predictable. When exposure to the chemical of interest overshadows concurrent exposures to other chemicals, one may assume that the biomarker is originated from one chemical. When exposures to other chemicals are also significant, one may use the predetermined environmental concentration ratio as input for a pharmacokinetic model to estimate the relative contribution of each parent chemical to the output biomarker concentration by tracking the time course of the metabolite from each parent chemical. However, if the ratio of multiple chemicals varies spatially and temporally because of different rates of production/use and environmental degradation, it will be necessary to collect more environmental measurements and/or use a fate and transport model in conjunction with an exposure model to estimate the ratio of different parent compounds' presence in the environment as a function of time and space. Once the fate and transport and exposure pathways are characterized, the uncertainty in the environmental concentration of the parent chemicals is reduced, and such information then can be used as input for a pharmacokinetic model to estimate the dose-biomarker relationship. On the other hand, if the metabolite, rather than the parent compound, is the toxic moiety, a nonspecific biomarker may be advantageous in that it integrates exposure to all parent chemicals within a single measure. Thus, the necessary approach for establishing exposure-biomarker relationship is dictated largely by the nature of exposure conditions, as well as the toxic mechanism of action.

¹⁰ Taylor et al. (2001). A Mixture Model for Occupational Exposure Mean Testing with a Limit of Detection. *Biometrics*, 57(3), 681-688.

5.6 Stereochemistry of biomarkers

Many environmental chemicals and biologically relevant molecules are “optically active” or chiral by nature.

Anthropogenic (e.g., synthetic pesticides) chiral chemicals often are synthesized as racemic mixtures, containing $2n$ isomeric components (where n = number of asymmetric or stereogenic centers, atoms with four different substituents). Enantiomers are stereoisomers that are nonsuperimposable mirror images of one another and have the same inherent physical-chemical properties (i.e., boiling point, vapor pressure, and molecular weight), whereas diastereomers and nonsuperimposable nonmirror images have similar but nonequivalent physicochemical properties, making them separable. Most chiral natural products (e.g., sugars, amino acids, lipids, and nucleic acids) exist as an enriched single isomeric form (i.e., homochiral). Many anthropogenic chemicals (e.g., pesticides and therapeutic drugs) may be designed or formulated to favor one biologically active racemate over the other to improve efficacy. Known enrichment/enhancement factors of stereoisomeric mixtures (natural versus anthropogenic) can provide a simple basis for differences in exposure when considering multimedia environmental factors (e.g., biological degradation pathways). However, molecular interactions involving enantiomers with endogenous molecules usually have different binding kinetics (e.g., affinities) with enzymes involved in metabolism or interactions with target ligands. Therefore, chirality may be expected to impact

exposure through ADME and, in turn, stereospecific differences in risk. Although consideration of chirality may seem to complicate exposure and risk assessments, consideration of the impact of chirality (or stereochemistry) on exposure and risk assessment is unavoidable. Because enantiomers can undergo reactions and metabolize differently in the body, chiral biomarkers may be used as unique markers that can be related back to exposure. By characterizing stereoselectivity in exposure for parent compounds and metabolites, additional information can be gleaned about exposure pathways and internal chemical disposition that cannot be obtained from remedial assessments based on achirality or two dimensional planar assumptions. Ultimately, boundary conditions between tissue distribution, metabolism and toxicity require knowledge of individual isomeric fate in the same way that mixtures of multiple chemicals with uniquely defined kinetics cannot be reduced by simple additive action as additional mass of each single chemical under the rubric of aggregate exposure and cumulative risk assessment.

6.0 Future Directions for Research on Biomarkers of Exposure

The future directions for research on biomarkers conducted in NERL will support several new research programs in the Office of Research and Development (ORD): Air, Climate, and Energy (ACE), Sustainable and Health Communities (SHC), and Chemical Safety for Sustainability (CSS). More specifically, one of the eight topics in CSS is “Biomarkers.” Currently, the linkage and translation of exposure and hazard data into human or ecological risk are conducted independently, which can often lead to data gaps and scientific uncertainties. One of the promising tools for linking different elements along the source-to-outcome continuum to understand the public health implications of exposure to environmental chemicals is biomarkers. Thus, the overall goals of the biomarkers research are (1) to develop the scientific knowledge and tools that will improve the use of biomonitoring data in both single and multiple chemical risk assessment and risk management decisions and (2) to improve our understanding of the fundamental processes and linkages along the exposure-dose-effects continuum that lead to risk.

Research in the biomarkers research topic will be led by scientists in NERL and the National Health and Environmental Effects Research Laboratory (NHEERL) and is organized around two projects outlined below.

1. Project 1 will identify biomarkers/bioindicators¹¹ and approaches of interpretation in the context of establishing exposure to dose to outcome linkages. With biomarkers/bioindicators linkages defined, this project will contribute to Project 2 that uses systems models for predicting adverse health and environmental effects of human and wildlife exposures.
2. Project 2 will evaluate the predictive models and develop robust tools for monitoring exposure and effects in clinical, epidemiological, and ecological field studies. These efforts will be coordinated with other CSS, ACE, and SHC topics to identify and understand the most important exposure sources, routes, and pathways for high-priority chemicals for human and wildlife species and how exposure is related to adverse outcome.

¹¹ Bioindicators are defined as measurements of biochemical or physiological changes within an organism that reflect biological responses.

For NERL, the research direction will focus in the following three areas.

1. Data collection and analysis
 - a. Develop a knowledge base of novel and existing biomarkers of exposure for high-priority and high-interest emerging chemicals
 - b. Develop or improve measurement and analytical methods for environmental and biological samples
 - c. Perform observational studies to collect data on environmental and biomarker concentrations, human time/location activities, and product/chemical use patterns
 - d. Develop and identify new biomarkers of exposure that are better indicators of exposure
2. Predictive modeling tools
 - a. Develop and apply *in silico* models to estimate inherent and derived chemical properties based on chemical structures for informing the selection of proper biomarkers
 - b. Evaluate the correlation among empirical environmental and biomarker data using statistical models
 - c. Describe the exposure-biomarker relationship using pharmacokinetic models
 - d. Use *in vitro* data or *in silico* models to estimate pharmacokinetic data for provisional or screening-level pharmacokinetic models
3. Integrated research
 - a. Integrate environmental media measurements, human activity observations, and other exposure factors with pharmacokinetic models for linking exposures to biomarkers
 - b. Identify normal ranges of “probative” biomarkers and quantify resiliency and shifts in homeostasis using biomarkers
 - c. Evaluate variability and susceptibility using biomarker measurements



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