Title:
A biomonitoring framework to support exposure and risk assessments

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Abstract

Background: Biomonitoring is used in exposure and risk assessments to reduce uncertainties along the source-to-outcome continuum. Specifically, biomarkers can help identify exposure sources, routes, and distributions, and reflect kinetic and dynamic processes following exposure events. A variety of computational models now utilize biomarkers to better understand exposures at the population, individual, and sub-individual (target) levels. However, guidance is needed to clarify biomonitoring use given available measurements and models.

Objective: This article presents a biomonitoring research framework designed to improve biomarker use and interpretation in support of exposure and risk assessments.

Discussion: The biomonitoring research framework is based on a modified source-to-outcome continuum. Five tiers of biomonitoring analyses are included in the framework, beginning with simple cross-sectional and longitudinal analyses, and ending with complex analyses using various empirical and mechanistic models. Measurements and model requirements of each tier are given, as well as considerations to enhance analyses. Simple theoretical examples are also given to demonstrate applications of the framework for observational exposure studies.

Conclusion: This biomonitoring framework can be used as a guide for interpreting existing biomarker data, designing new studies to answer specific exposure- and risk-based questions, and integrating knowledge across scientific disciplines to better address human health risks.

Key Words:
biomonitoring; biomarkers; exposure science; exposure assessment; risk assessment

Abbreviations:
BR biomarker, biologically-relevant biomarker; BR dose, biologically-relevant dose

1.0 Introduction

The U.S. Environmental Protection Agency (USEPA) and other organizations use risk assessments to determine whether actions should be taken to protect public health (USEPA, 2009). Risk assessments are based on the concept that:

$$\text{Risk} = \text{Hazard} \times \text{Exposure}$$

For a given chemical, toxicity testing is used to identify a hazard and to establish a quantitative relationship between administered dose and the incidence of health effects. This dose-response relationship is then used to develop an acceptable human exposure level (*e.g.*, a reference dose [RfD]). Next, exposure assessments identify the source(s), route(s), and magnitude of human exposure. The risk of an adverse outcome is then determined by comparing observed or estimated exposures to the acceptable level. Finally, information on sources and routes of exposure are used to identify effective mitigation strategies.

In the past, blunt tools have generally been used for risk assessments – that is, high-dose animal toxicity tests and screening-level exposure assessments. With these tools, the links between exposure and health outcome are highly uncertain. Biomonitoring, because it is close to the center of the source-to-outcome continuum, should better inform these linkages and reduce the associated uncertainties. However, applications of biomonitoring in exposure and risk assessments are limited by a lack of guidance on data use and interpretation.

This article presents a modified source-to-outcome continuum that provides a framework for biomonitoring to support exposure and risk assessments. The framework includes five tiers that can be used to answer specific exposure- and risk-based questions. The overall intent is to provide guidance for interpreting existing biomarker data, designing new biomonitoring studies

to efficiently answer targeted research questions, and synthesizing relevant information across scientific disciplines to address human health risks.

2.0 Discussion

2.1 A modified source-to-outcome continuum

Figure 1 illustrates a modified source-to-outcome continuum that highlights traditional components of exposure science and contemporary components of health effects science. Biomarker measurements are central to the continuum, and therefore link the exposure and health effects components.

Definitions of components within the modified source-to-outcome continuum (shown in Fig. 1) include the following:

- ***Environmental measurements*** are observed concentrations of stressors in environmental media. While stressors can be biological, physical, or even psychosocial, this article focuses on chemical stressors.
- ***Exposure models*** mathematically combine environmental measurements with human activities and other exposure factors to generate exposure estimates.
- ***Exposure estimates*** are route specific (*e.g.*, inhalation, ingestion, or dermal exposure) and quantify the mass of a chemical that comes into contact with a human over time.

- ***Kinetic models*** mathematically describe the movement of a chemical through the body; that is, its movement into the body (*absorption*) following exposure, *distribution* to various tissues, *metabolism* by various processes, and ultimate *elimination* from the body (these kinetic processes are collectively called "ADME").
- ***Dose estimates*** are based on exposure estimates and kinetic processes, and quantify the integrated (over time) mass of a chemical inside the body.
- ***Biomarker measurements*** are observations of chemicals, chemical metabolites, and target molecules (*e.g.,* chemical adducts) in biological media (*e.g.,* blood, breath, and urine) that, for the purposes of this paper, reflect exposure events and dose.
- ***Biologically-relevant (BR) dose estimates*** are based on dose estimates and kinetic processes, and quantify the amount of the dose at a specific target (inside a human) that is associated with key events in a disease process (*e.g.*, the amount of a neurotoxin in the brain, or the amount of a genotoxic metabolite that interacts with genetic material). (This definition follows from that of "biologically relevant exposures" given by Birnbaum (2010)).
- ***Dynamic models*** mathematically describe the impacts of the BR dose on biological systems (*e.g.*, enzyme inhibition from a neurotoxin, or DNA damage from a genotoxic metabolite) and are used to predict BR biomarker levels.
- ***BR biomarker measurements*** are observations of chemicals/molecules in biological media that reflect (directly or indirectly) the BR dose (*e.g.,* blood enzyme levels to reflect the BR dose of a neurotoxin).
- ***Statistical models*** compare observations of random variables for hypothesis testing. For example, statistical models can evaluate associations between environmental and

biomarker measurements, and biomarker and BR biomarker measurements, as well as the effects of covariates (*e.g.*, age, gender, and human activities) on these associations.

Figure 1 shows that components of the modified source-to-outcome continuum align along two planes: (1) measured values (*i.e.*, environmental, biomarker, and BR biomarker measurements) shown with blue boxes; and (2) estimated values (*i.e.*, exposure, dose, and BR dose estimates) shown with red triangles. While measured values are subject to uncertainty based on assay precision, estimated values depend on measurements, observations, *and* model parameters, and are therefore subject to greater uncertainty. Biomarker measurements, which are at the center of the continuum, can help reduce uncertainties in estimated values as described in the following sections.

Our proposed biomonitoring framework has five tiers that describe different uses for biomarkers with different levels of complexity. Tier 1 considers only biomarker measurements, and subsequent tiers consider additional measurements, models, and estimated values. Simple theoretical examples are given for each tier 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 allow continuity from one tier to the next, and to simplify the interpretation and discussion. Example biomarkers for each tier are assumed to be measurable using reliable sampling and analytical methods, and to reflect exposure to environmental chemicals. The specific criteria for biomarker selection and use are not the focus of this article and can be found elsewhere (Metcalf and Orloff, 2004; NRC, 1987; NRC, 1991; NRC, 2006; Sobus et al., 2010a).

2.2 Biomonitoring framework tiers

2.2.1 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:

- Who is exposed?
- What are the exposure trends?
- Which chemicals should be prioritized for higher-tier analyses?

As shown in Figure 2, biomarker measurements are the only requirement for a Tier 1 analysis. Specifically, cross-sectional biomarker measurements are used to evaluate exposures across populations, and longitudinal biomarker measurements are used to evaluate exposure trends within a population. To demonstrate these uses, two theoretical examples are given in Figure 2.

In Example 1, the two distributions represent biomarker measurements that have been separated into groups for hypothesis testing; example groups could include those separated by gender, geographical area, age (*e.g.*, < 18 years old vs. ≥ 18 years old), or product use. All other things being equal, observed differences between grouped measurements indicate an effect of the grouping variable on biomarker levels, and suggest exposure differences between the groups.

In Example 2, longitudinal biomarker measurements for a population decrease over time, suggesting a decrease in exposure levels. Trends over time can indicate a change in the exposure source (*e.g.*, de-registration 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 are generally needed to pinpoint the cause of a trend. For examples of Tier 1 analyses, see Barr et al. (2010), Naeher et al. (2010), Calafat et al. (2010), Pirkle et al. (2006), and Sobus et al. (2009b; 2009c).

2.2.2 Tier 2: Biomonitoring to support exposure assessment

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

- What are the likely exposure sources?
- What are the likely exposure routes?

As shown in Figure 3, Tier 2 analyses consider paired environmental and biomarker measurements at the subject level, and focus on statistical comparisons of these data. A graph in Figure 3 shows a regression of "spot" biomarker measurements (one observation per subject) 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, environmental measurements in this example were concentrations of a chemical in food, and 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. Potentially, the results could point to food or 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, gender, age, and ethnicity) 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.*, including environmental measurements of different media to identify additional exposure routes), or of a higher-tier analysis. For examples of Tier 2 analyses, see Egeghy et al. (2005), Thomas et al. (2010a; 2010b), and Sobus et al. (2009a)

2.2.3 Tier 3: Biomonitoring to support risk assessment

Tier 3 analyses of biomarker data can be used to support risk assessments since they can answer the following questions:

- What are the important exposure factors?
- What are the likely exposure 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 considerations for exposure. Here, exposure is directly linked to environmental measurements via exposure models, and indirectly linked to biomarker measurements via statistical models (*e.g.*, multiple regression models) that consider

environmental measurements, human activities, and other exposure factors. Statistical models are used to identify important predictors of exposure and can therefore inform exposure calculations. Since exposure estimates are comparable to acceptable levels based on animal studies, Tier 3 analyses can place biomarkers into a risk context.

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 estimates based on dietary ingestion would be appropriate (but not necessarily accurate) and comparable to acceptable levels for risk evaluation. However, given added information (such as human activities data), it would be possible to explain more biomarker variance, thus increasing the accuracy of the exposure estimates. The graph in Figure 4 shows a regression of biomarker levels on covariate-adjusted environmental levels. Here, the adjusted environmental levels reflect for each individual the combined effects of food concentration, food consumption (total mass), and other covariates. 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. Thus, the significant covariates identified through statistical analyses could be considered, along with the food measurements, to improve exposure estimates. For examples of Tier 3 analyses, see Morgan et al. (2007; 2005; 2008), Wilson et al. (2007), and Tulve et al. (2010).

2.2.4 Special considerations for biomarker variance components

The frequency and duration of exposure, timing of sampling, and rates of kinetic processes (*i.e.*, ADME) can impact biomarker interpretation with respect to exposure and dose. Regression

slopes and R^2 values in Tier 2 and Tier 3 analyses can reflect the magnitude of these impacts. However, it is difficult to quantify these effects without measuring biomarker concentrations over time. Longitudinal studies (with repeated measurements) can partition biomarker variance into that which is observed between subjects and that which is observed for a given subject over time. The respective magnitudes of these variance components can inform the importance of timed events and kinetic processes in biomonitoring studies, as demonstrated below.

Figures 5A and B show repeated biomarker measurements of individuals from two theoretical groups. Both figures show 10 consecutive measurements of 50 subjects, with the first measurements made at 6:00 am and the final measurements at midnight (12:00 am) on the same day. In Figure 5A, biomarker measurements vary slightly over time (small within-person variance) and are distinguishable between individuals (large between-person variance). These observations suggest that individuals have different exposure/dose levels and that kinetic processes occur slowly. Figure 5B shows large within-person variance in biomarker levels and considerable overlap across individuals (small between-person variance), suggesting similar exposure/dose levels between individuals and rapid chemical uptake and elimination.

Three example regressions of dose estimates on biomarker levels are given in both Figures 5A and B; here dose is approximated for each individual as their average biomarker level across all 10 measurements. In Example 1, dose is regressed on randomly selected spot biomarker levels; this simulates studies where one random biomarker measurement is made for each subject. Example 2 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, dose is regressed on the average of three randomly selected measurements;

298 this simulates studies where repeated measurements are made for each subject, and the
299 measurements (or the biological samples themselves) are pooled (averaged) prior to analysis.
300 All three examples in Figure 5A show very similar slopes (ranging from 0.92 to 1.0) and
301 R^2 values (ranging from 0.93 to 0.99). These results indicate that sampling strategy has little
302 impact on biomarker interpretation when the between-person variance is large compared to the
303 within-person variance. Specifically, these results suggest that the biomarker measurements
304 from each of the three sampling examples could be used to accurately and precisely estimate
305 dose levels (given the approximation of "dose" using average biomarker levels).
306 In contrast, the examples in Figure 5B indicate increased impacts of sampling events on
307 biomarker interpretation when the within-person variance is large compared to the between-
308 person variance (slope range: 0.23 – 0.82; R^2 range: 0.22 – 0.76). Example 3, using the average
309 of three random measurements, shows the strongest association suggesting that multiple
310 measurements are needed when longitudinal data are highly varied. Results also show that spot
311 biomarker measurements, collected randomly (Example 1) or at a fixed time (Example 2), can
312 severely underestimate dose levels (slopes << 1) in these instances. (The importance of slope
313 attenuation with measurement error, defined as "attenuation bias", is further described by Lin et
314 al. (2005), Rappaport and Kupper (2008), and Sobus et al. (2010b)). Taken together, the
315 examples in Figures 5A and B demonstrate the need to (1) understand biomarker variance
316 components via repeated observations, and (2) sample more frequently when the within-person
317 variance is large compared to the between-person variance; this allows better estimation of the
318 "true" average biomarker level as an indicator of exposure/dose.
319
320 **2.2.5 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 (1) exposure and dose, (2) dose and biomarker levels, and (3) dose and BR dose. Linking the external environment to internal dose and biomarker levels is a primary goal of exposure science (Sheldon and Cohen Hubal, 2009). Therefore, Tier 4 analyses represent a general endpoint for exposure science and a starting point for health effects science. Furthermore, since the BR dose estimate is the final output from a Tier 4 analysis, it can be considered as the final output from exposure research, as well as a useful input for health effects research (Pleil and Sheldon, 2010).

Using environmental measurements, exposure factors, and exposure and kinetic models, Tier 4 analyses can answer the following questions for exposure and risk assessments:

- What is the importance of each exposure route?
- What are the best estimates of exposure and dose?
- What are the likely BR dose levels?

In Tier 3, exposure estimates and biomarker measurements were not directly linked. Rather, results from statistical models were used as support for exposure 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 with a short biological half-life following three dietary exposure events (*e.g.*, breakfast, lunch, and dinner). Assuming a well-parameterized and calibrated model, good agreement between predicted and observed values support that diet is the primary exposure source and help validate exposure estimates. Overestimation of the observed values would suggest incorrect exposure estimates, whereas underestimation could suggest additional exposure routes or endogenous sources of the biomarker. In these situations, exposure estimates could be reconstructed to be consistent with observed values. (For methods and examples of exposure reconstruction, see Kim et al. (2007), Tan et al. (2006), and Clewell et al. (2008)).

Given the appropriate model structure and parameters, the same kinetic models used to predict biomarker levels may be used to predict the BR dose. Example 2 in Figure 6 extends Example 1 and shows predicted levels at a target over time. In this theoretical example, the parent chemical is neurotoxic, the target is the brain, and the predicted values are concentrations of the parent chemical in the brain. Here, the health risks of the predicted values could be evaluated using results of health effects studies. Specifically, the area under the target-level curve (AUC_{target}, which is the time-integrated BR dose), or the maximum concentration at the target, could be interpreted given some knowledge of the BR dose-response relationship. For an example of a Tier 4 analysis, see Hore et al. (2006).

2.2.6 Tier 5: Biomonitoring to advance exposure and risk assessments

As shown in Figure 7, Tier 5 analyses of biomarker data include all components of the source-to-outcome continuum, and predict both biomarker and BR biomarker levels for comparison to measured values. These comparisons allow Tier 5 analyses to answer the following research questions:

- What are the best estimates of BR dose?
- What are the likely impacts of exposure on health risks?
- What other factors may affect health risks?

In Tier 4 analyses, BR dose is estimated using kinetic models, and interpreted using knowledge of the BR dose-response relationship. Since BR dose estimates are not confirmed with measured values, there is uncertainty in model predictions. Tier 5 analyses further utilize kinetic/dynamic models to predict BR biomarker levels based on the BR dose estimates. Comparison of the predicted and observed BR biomarker levels can then reduce uncertainties in the BR dose estimates.

Example 1 in Figure 7 shows predicted versus observed levels of a BR biomarker. This extends the examples in Figure 6 where the brain was a target tissue, and the stressor was a chemical neurotoxin from food. In this example, blood enzymes are the BR biomarkers and act as surrogates for brain enzymes (*e.g.*, cholinesterase). Combined 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 predicted and observed values indicates an accurate estimation of BR dose. Thus, the BR dose estimate could be used to inform health risks

from exposure. However, poor agreement between predicted and observed BR biomarker levels would suggest an incomplete understanding of kinetic/dynamic processes *in vivo*. 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 placing BR dose estimates into a risk context.

Statistical comparisons of biomarker and BR biomarker measurements are also used in Tier 5 analyses to elucidate health risks from exposure. For example, *in vivo* dose-response associations can be informed using regressions of BR biomarker levels (representing response) on biomarker levels (representing dose). Modifiers of the *in vivo* dose-response relationships can also be observed by studying covariates such as age, gender, personal and 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 activities decreased with increasing adjusted biomarker levels. In other words, biological function was suppressed given elevated dose levels. This observation (specifically, the slope of the regression line), as well as the model results for covariates (coefficients and p-values), could help inform both exposure and susceptibility effects on BR dose, and by extension, health risks. For an example of a Tier 5 analysis, see Garabrant et al. (2009).

3.0 Conclusions

Biomonitoring data can be used in many different ways; applications can be as simple as documenting a population-based change in exposure, or as complex as linking the source-to-outcome progression through empirical data and sophisticated models. In this article, we created a biomonitoring framework to demonstrate how measurements, models, and model estimates are used together to answer specific exposure- and risk-based questions. In doing so, we presented a tiered approach that categorizes the uses of biomarker data in the presence or absence of corresponding information. (A summary of the uses and requirements of the five biomonitoring tiers is given in Table 1). This tiered approach does not imply a hierarchy of biomonitoring research based on value; that is, it does not rank analyses from "least useful" to "most useful". Rather, it poses a logical structure to what is often a complex web of information. This structure will help researchers (1) conceptualize sampling and analysis approaches when designing targeted studies, and (2) weigh the costs (personnel, instrumentation, and time) and benefits (ability to answer specific questions) of proposed studies, with the goal of maximizing public health benefits.

Simple theoretical examples were given throughout the text to articulate the tiered biomonitoring approaches. While we recommend using these examples as a guide for interpreting existing data sets and for designing new studies, we caution that these examples were simplified for demonstration purposes. That is, they did not address some common challenges of biomonitoring studies, such as the needs to resolve non-specific biomarkers, overcome analytical detection limitations, and interpret urinary biomarkers that are affected by host hydration level (Albertini et al., 2006; Angerer et al., 2006; Barr and Angerer, 2006; Clewell et al., 2008; Needham et al., 2007; NRC, 2006; Sobus et al., 2010a). Also, the examples were generally geared towards evaluating short-term biomarkers of non-persistent chemicals,

433 particularly those given for Tier 4 and Tier 5 analyses; different approaches can be considered
434 when evaluating intermediate- and long-term biomarkers (Clewell et al., 2008; Rappaport and
435 Kupper, 2008). Finally, the examples assumed that analytical methods, mechanistic models
436 (*e.g.*, kinetic and dynamic models), and acceptable exposure levels exist for a given chemical of
437 interest. Complications arising from any one of these issues can hinder a biomonitoring analysis
438 and limit the use of individual biomarkers in research studies.

439 Given the potential limitations of biomonitoring studies, this framework lays a foundation
440 for identifying the key data and modeling gaps, and prioritizing research needs. For example,
441 this framework can help prioritize the needs for (1) empirical evidence to inform kinetic
442 parameters, (2) well-vetted exposure and kinetic models, (3) improved methods to measure
443 environmental concentrations, biomarkers, and BR biomarkers, and (4) robust datasets with
444 which to estimate biomarker variance components and to perform statistical analyses. In ensuing
445 articles, we will discuss current and proposed efforts to address these needs while keeping within
446 the structure of this framework. The ultimate goal of these efforts is to provide cohesive
447 guidance that informs future biomonitoring studies, and catalyzes biomarker use in exposure and
448 human health research.

449

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Figure captions:

Figure 1. A source-to-outcome continuum.

Figure 2. Requirements and examples of Tier 1 analyses of biomarker data. Grey objects are unavailable in a Tier 1 analysis. Example 1 demonstrates a cross-sectional analysis where cumulative percentile distributions of biomarker levels are compared across two groups. Example 2 demonstrates a longitudinal analysis where biomarker levels for one group are examined over time.

Figure 3. Requirements and an example of a Tier 2 analysis of biomarker data. Grey objects are unavailable in a Tier 2 analysis. The example graph shows a regression of spot biomarker measurements on corresponding environmental measurements with an R^2 of 0.3.

Figure 4. Requirements and an example of a Tier 3 analysis of biomarker data. Grey objects are unavailable in a Tier 3 analysis. Exposure estimates can be compared to acceptable levels determined from animal studies. The example graph shows a regression of spot biomarker measurements on corresponding covariate-adjusted environmental measurements with an R^2 of 0.6.

Figure 5. Impacts of sampling events on biomarker interpretation when between-person variance is large and within-person variance is small (A), and when within-person variance is large and between-person variance is small (B). In both (A) and (B), Example 1 shows dose regressed on randomly selected spot biomarker measurements, Example 2 shows dose regressed on end-of-day biomarker levels, and Example 3 shows dose regressed on the average of three randomly selected measurements. Here dose is approximated for each individual as their average biomarker level across all 10 measurements.

Figure 6. Requirements and examples of Tier 4 analyses of biomarker data. Grey objects are unavailable in a Tier 4 analysis. Example 1 compares predicted and observed levels of a chemical biomarker over time. Example 2 shows predicted target levels over time of the same chemical from example 1. Here, the area under the target-level curve (AUC_{target}) is the BR dose.

Figure 7. Requirements and examples of Tier 5 analyses of biomarker data. Example 1 compares predicted and observed BR biomarker levels over time. Example 2 shows a regression of BR biomarker measurements on covariate-adjusted biomarker levels.

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Table

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Table 1. Uses and requirements of the five biomonitoring tiers.

Tier	Primary uses	Measurements needed	Models needed	Estimated values
1	Exposure surveillance: Who is exposed? What are the exposure trends? Which chemicals should be prioritized for higher-tier analyses?	1) Biomarker	none	none
2	Supporting exposure assessment: What are the likely exposure sources? What are the likely exposure routes?	1) Environmental 2) Biomarker	1) Statistical	none
3	Supporting risk assessment: What are the important exposure factors? What are the likely exposure levels?	1) Environmental 2) Biomarker	1) Statistical 2) Exposure	1) Exposure
4	Exposure and risk assessment: What is the importance of each exposure route? What are the best estimates of exposure and dose? What are the likely BR dose levels?	1) Environmental 2) Biomarker	1) Statistical 2) Exposure 3) Kinetic	1) Exposure 2) Dose 3) BR dose
5	Advancing exposure and risk assessment: What are the best estimates of BR dose? What are the likely impacts of exposure on health risks? What other factors may affect health risks?	1) Environmental 2) Biomarker 3) BR biomarker	1) Statistical 2) Exposure 3) Kinetic 4) Dynamic	1) Exposure 2) Dose 3) BR dose

Figure1

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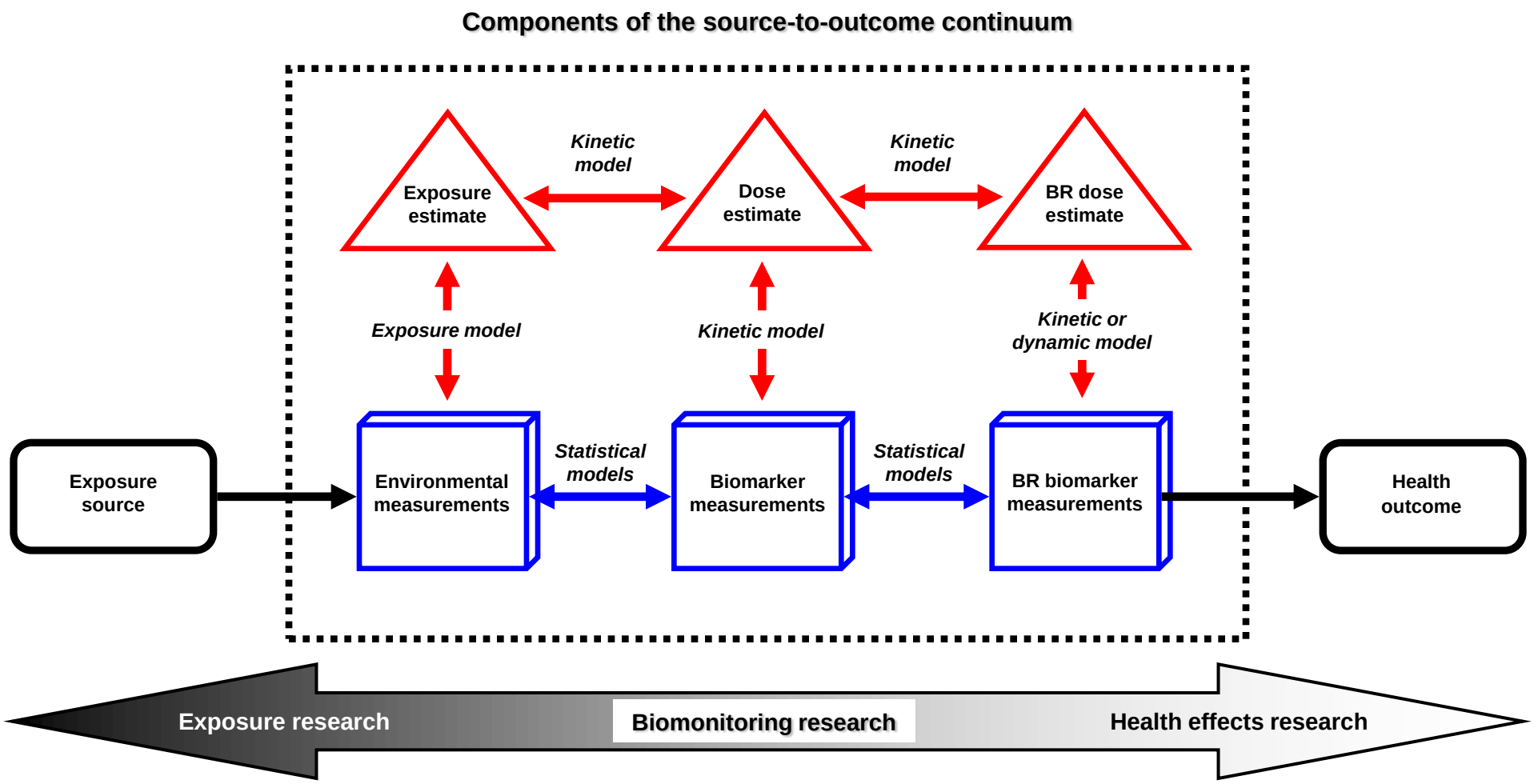

Figure1key

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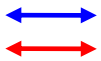
Symbol	Key	Parameter	Definition
	Estimated Value	1) Exposure estimate 2) Dose estimate 3) BR dose estimate	1) Estimated mass of a chemical that comes into contact with a human over time 2) Estimated mass of a chemical inside a human over time 3) Estimate amount of the dose at a specific target inside a human
	Measured value	1) Environmental measurement 2) Biomarker measurement 3) BR biomarker measurement	1) Observation of a stressor in environmental media that reflects a source 2) Observation of a stressor in biological media that reflects an exposure/dose 3) Observation of a stressor in biological media that reflects a BR dose
	Empirical model Mechanistic model	1) Statistical model (blue) 2) Exposure model (red) 3) Kinetic model (red) 4) Dynamic model (red)	1) Model that evaluates observed variables for hypothesis testing 2) Model that estimates exposure using environmental measurements and exposure factors 3) Model that describes how a stressor enters and is removed from a human 4) Model that describes the effect of a stressor on the human body

Figure2

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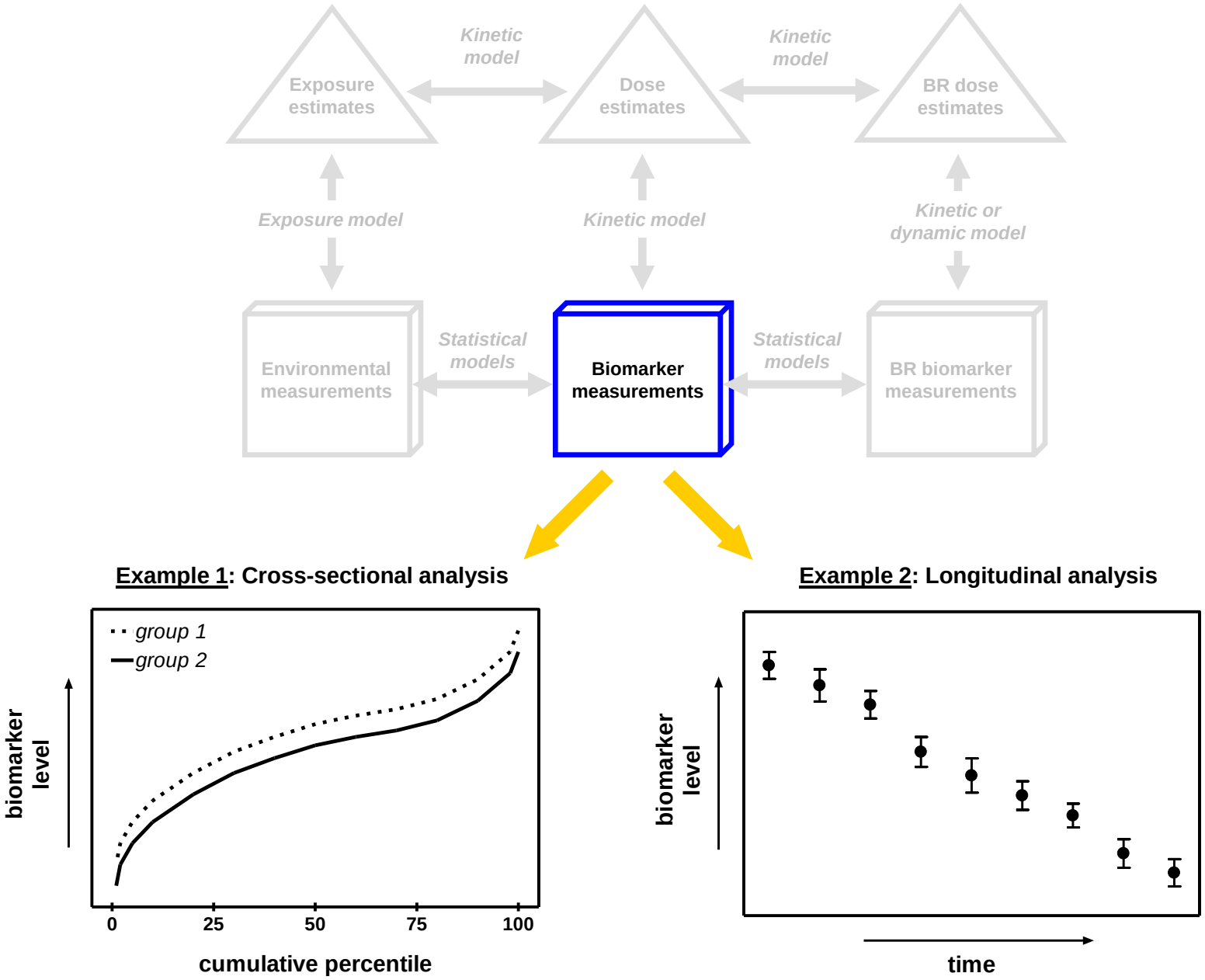

Figure3

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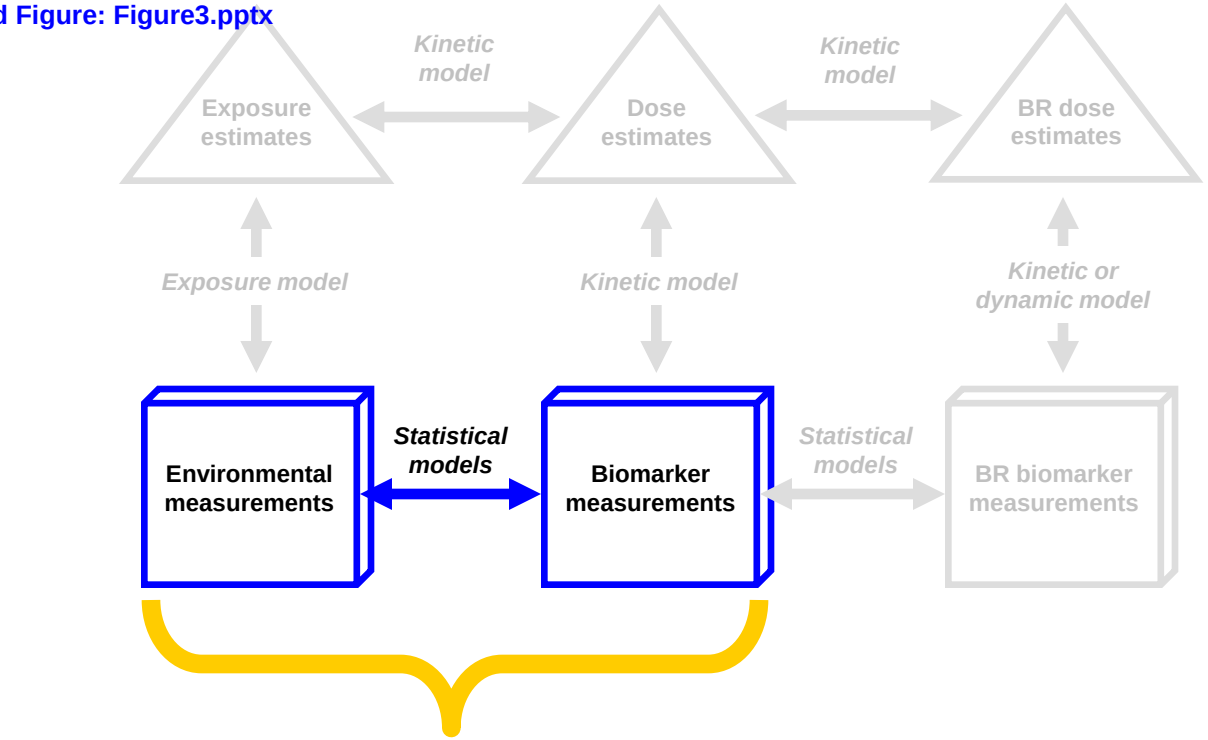

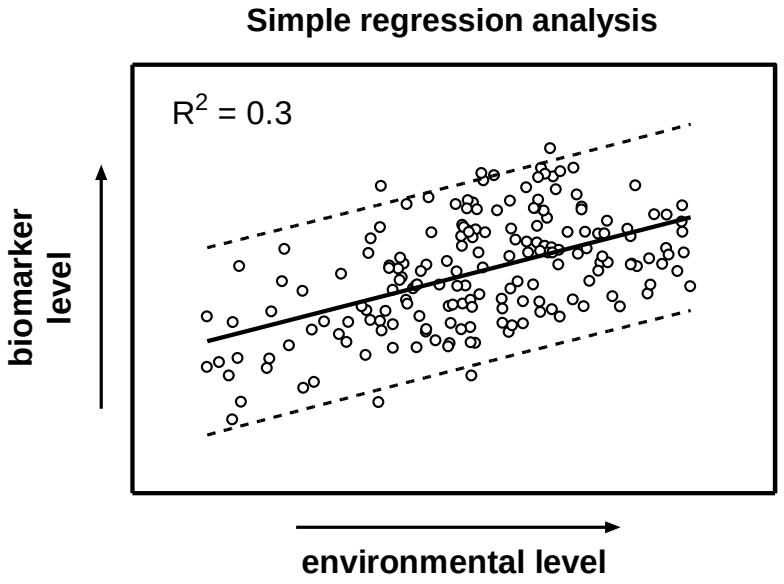

Figure4

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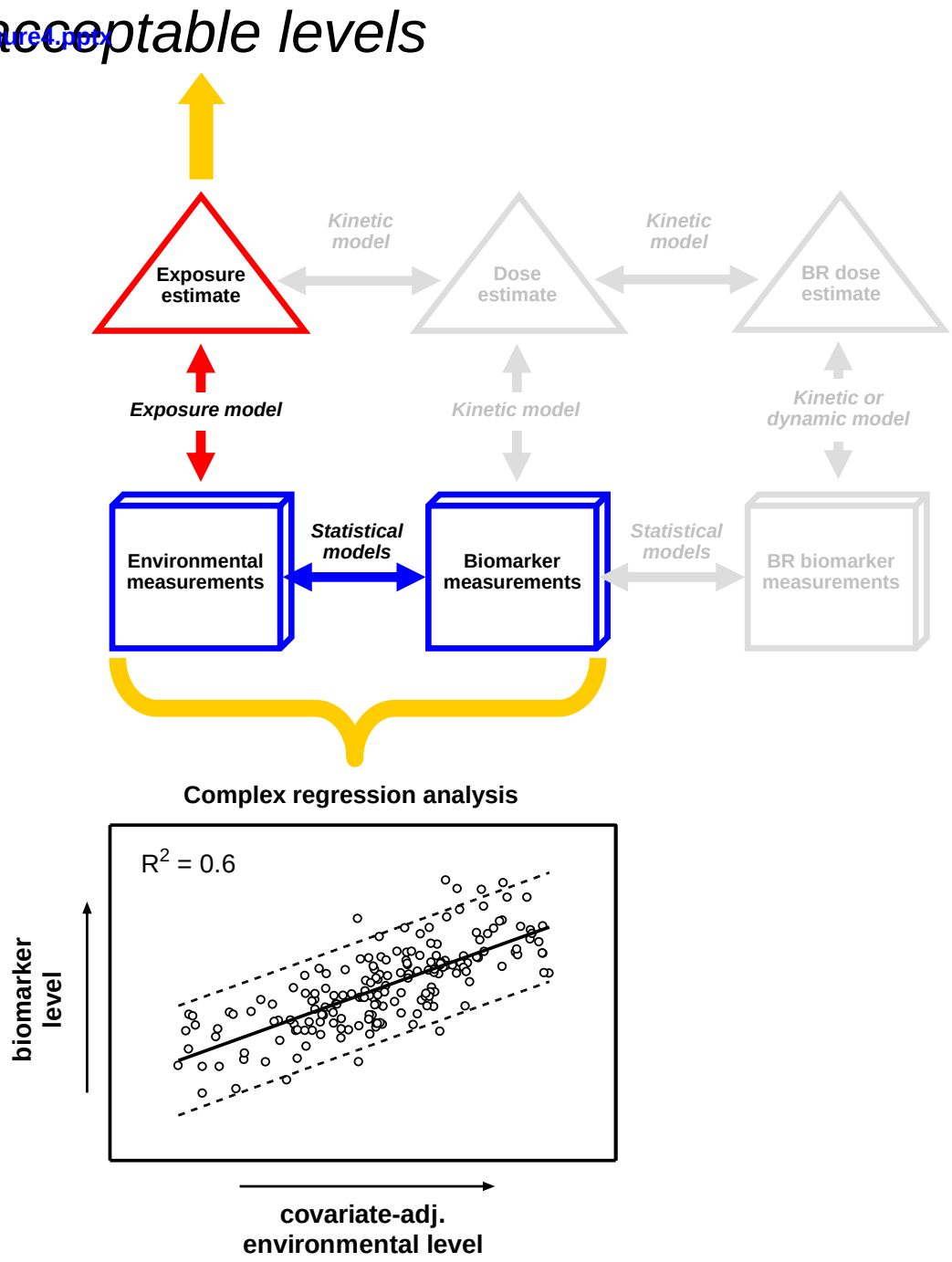

Figure5a

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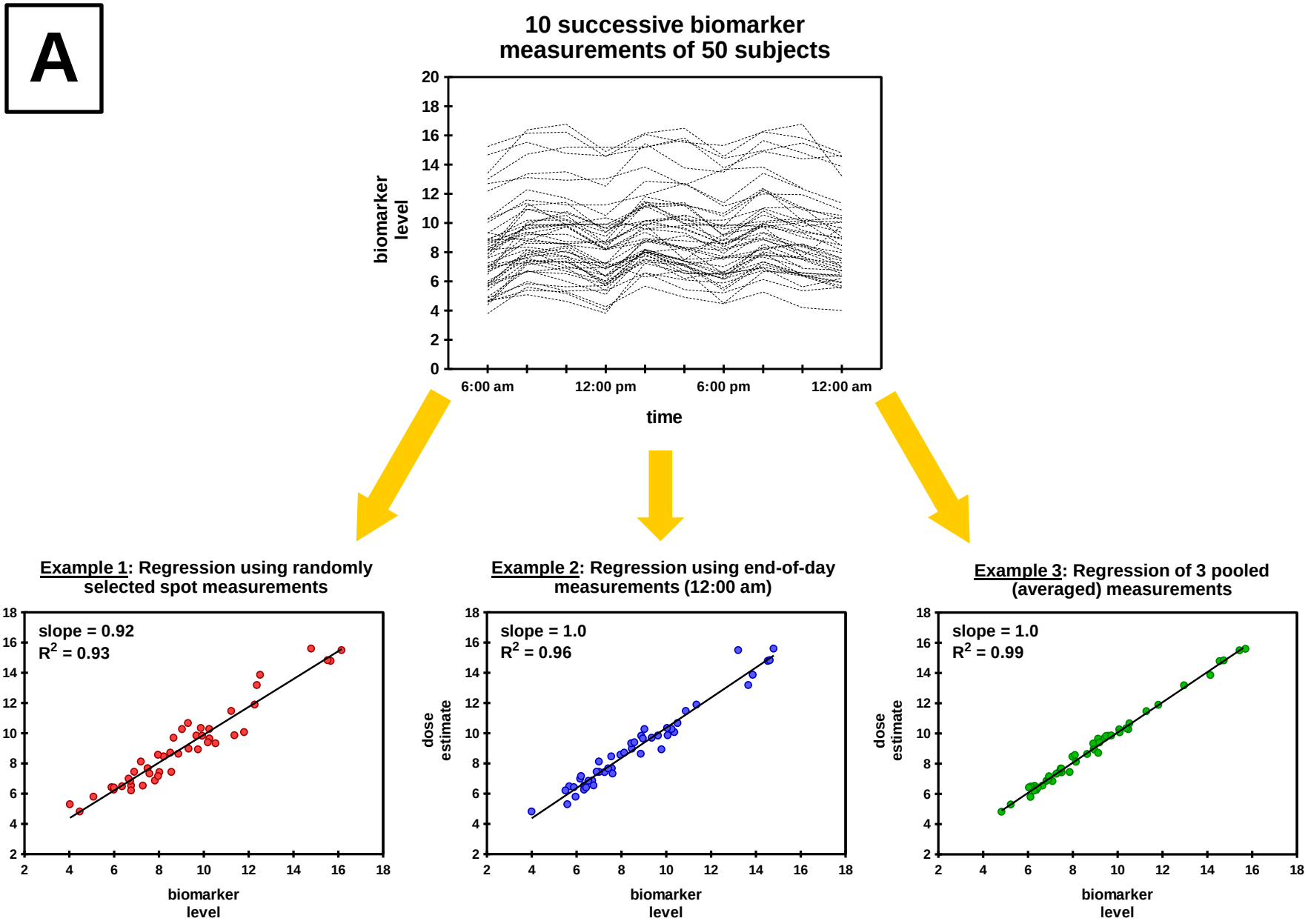

Figure5b

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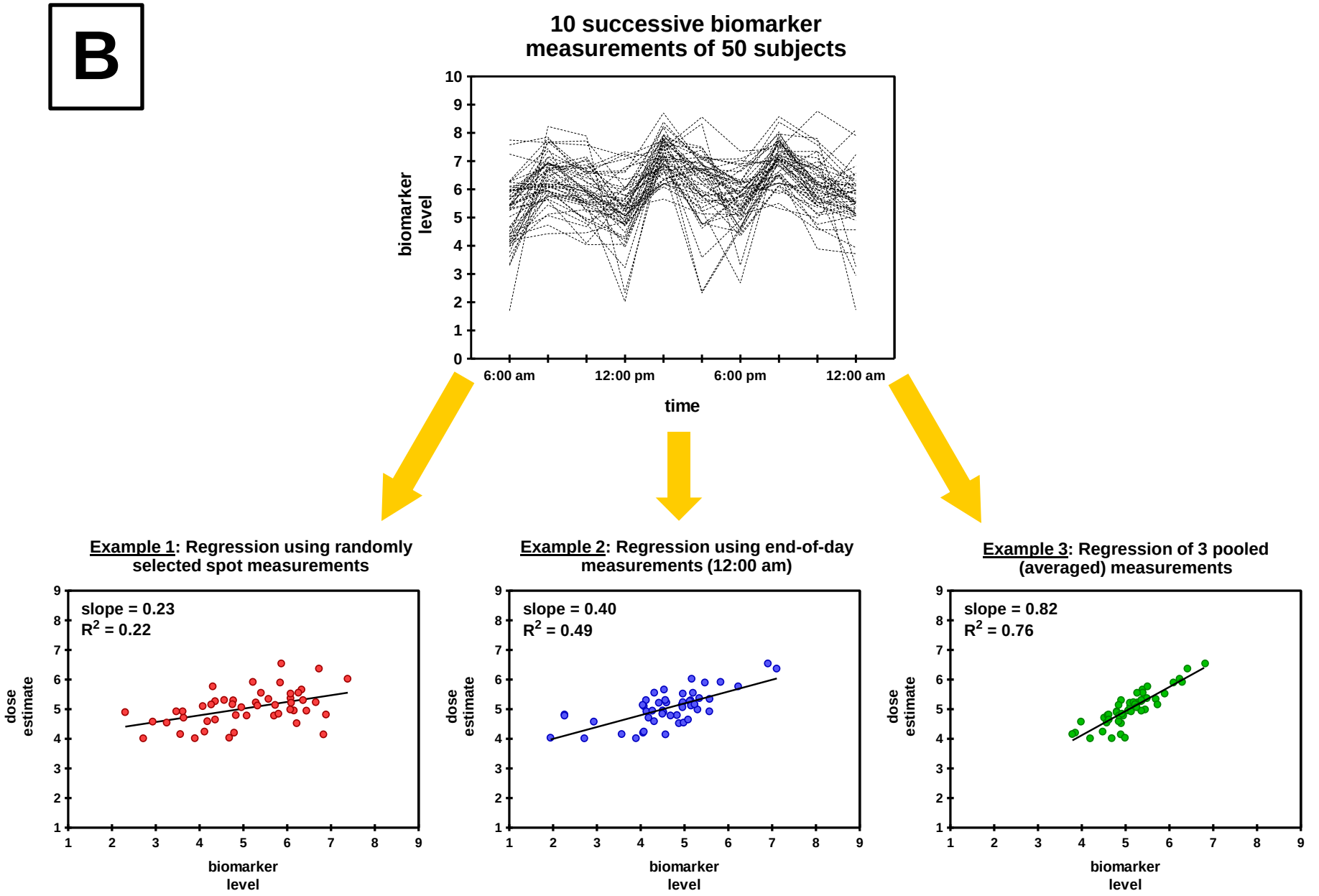

Figure6

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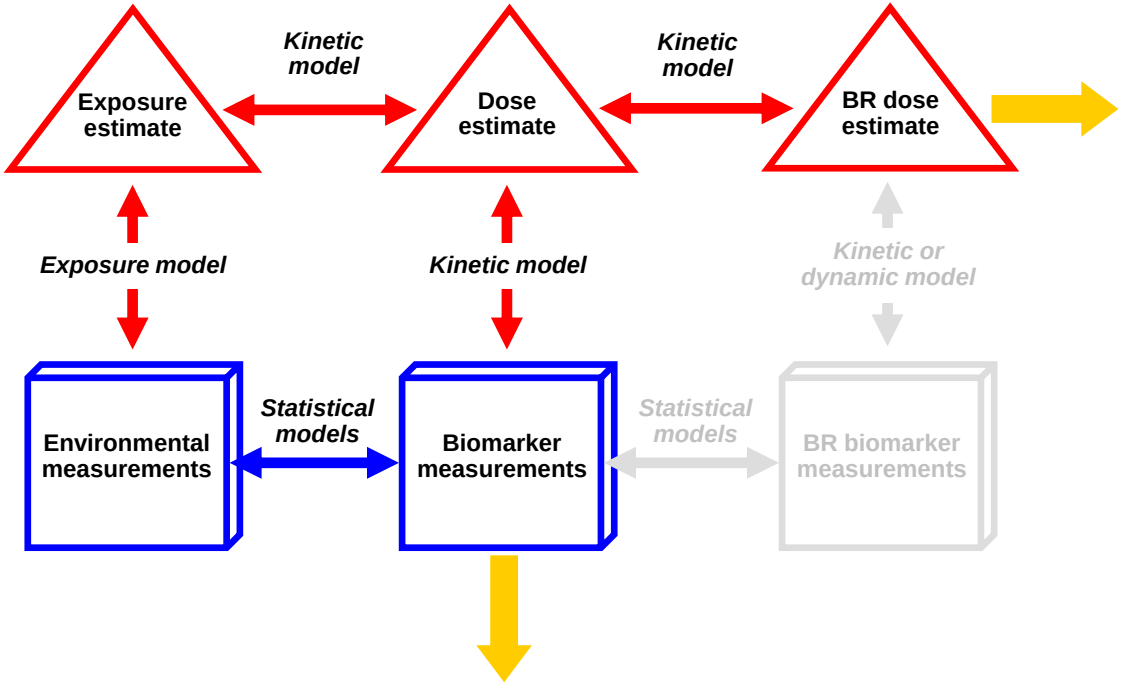
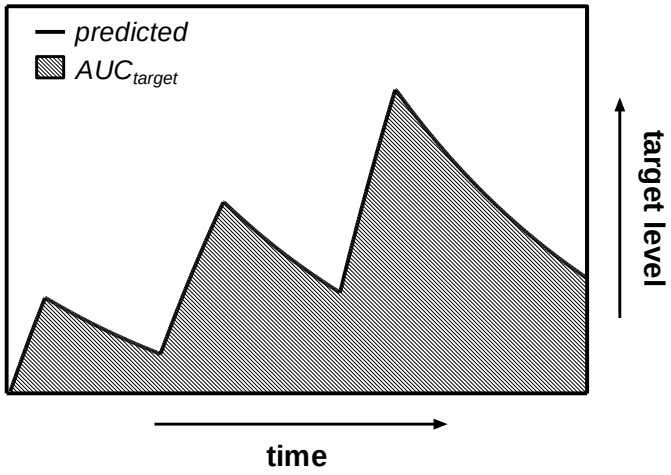


Example 2: Predicted target levels



Example 1: Predicted *vs.* observed biomarker levels

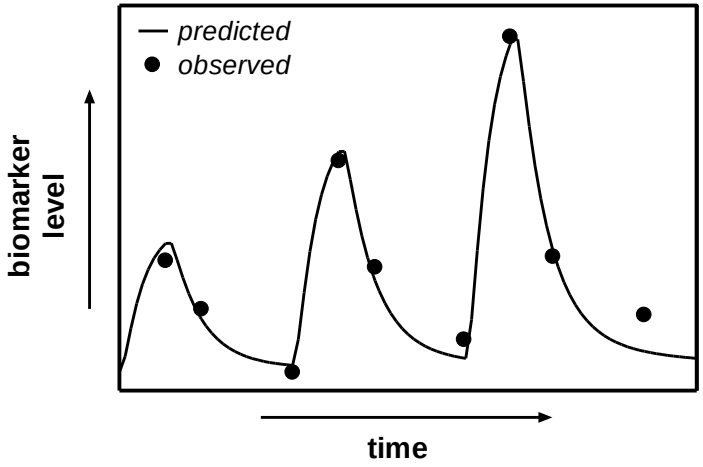


Figure7

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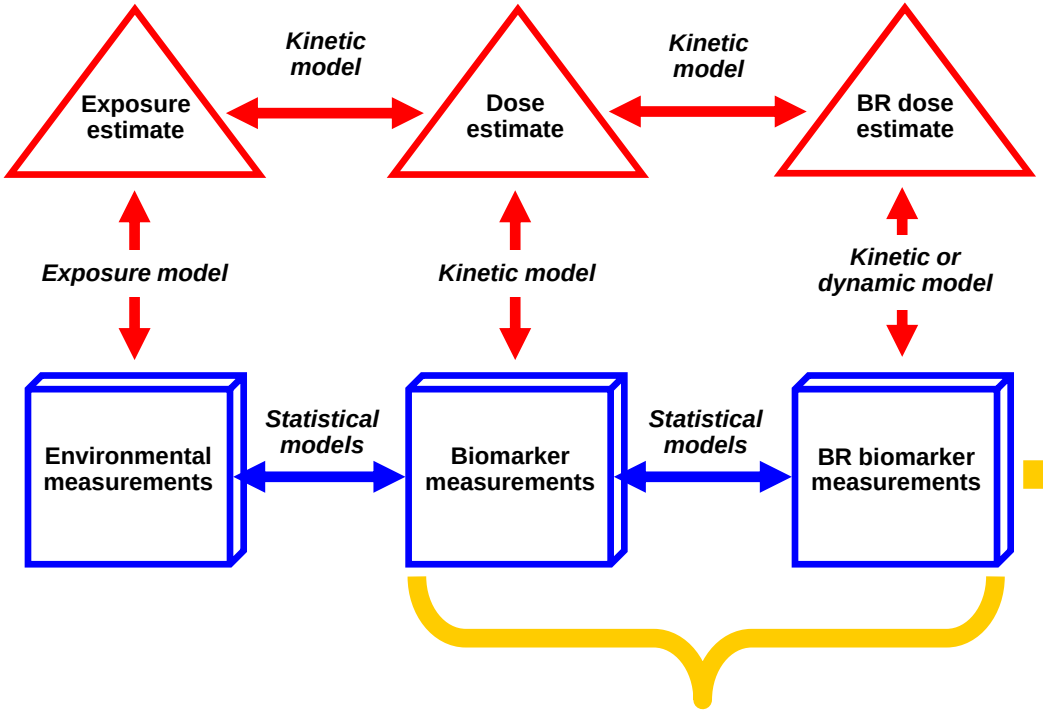

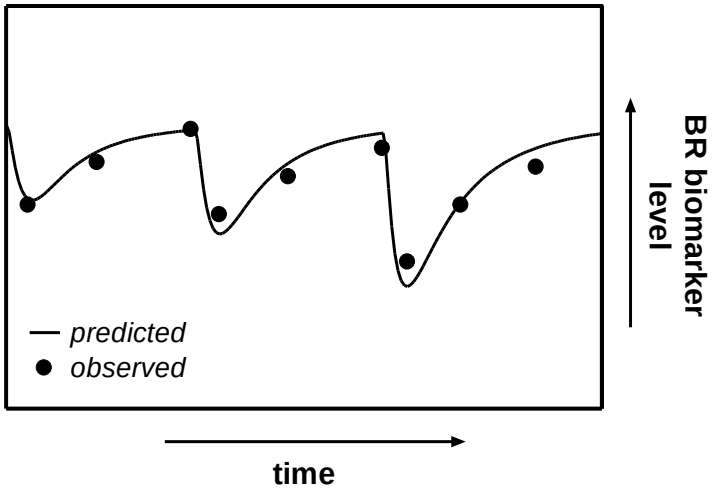

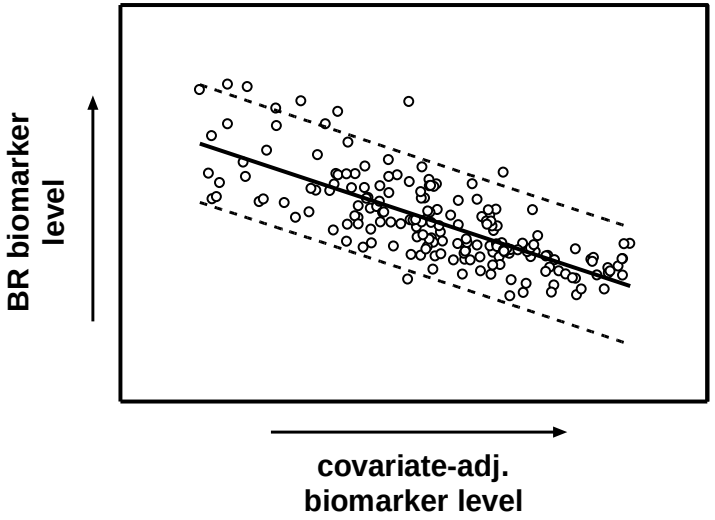

Highlights

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