

ASSESSING BIOMARKER USE IN RISK ASSESSMENT—A SURVEY OF PRACTITIONERS

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Advances in molecular epidemiology and mechanistic toxicology have provided increased opportunities for incorporating biomarkers in the human health risk assessment process. For years, the published literature has lauded the concept of incorporating biomarkers into risk assessments as a means to reduce uncertainty in estimating health risk. For all the potential benefits, one would think that markers of effective dose, markers of early biological effects, and markers of human susceptibility are frequently selected as the basis for quantitative human health risk assessments. For this article, we sought to determine the degree to which this evolution in risk assessment has come to pass. The extent to which biomarkers are being used in current human health risk assessment was determined through an informal survey of leading risk assessment practitioners. Case studies highlighting the evolution of risk assessment methods to include biomarkers are also described. The goal of this review was to enhance the implementation of biomarker technology in risk assessment by (1) highlighting successes in biomarker implementation, (2) identifying key barriers to overcome, and (3) describing evolutions in risk assessment methods.

Perera and Weinstein's 1982 vision of molecular epidemiology provided a structure featuring four categories of biomarkers: (1) internal dose, (2) biologically effective dose, (3) response, and (4) susceptibility (Perera & Weinstein, 1982). While initially focused on cancer and cancer prevention, an early goal was to incorporate molecular endpoints into epidemiological studies so that researchers "should be able to predict human risks more precisely than hitherto possible." Seventeen years later, Perera (2000) concluded that "what is currently needed is the timely translation of existing data into *risk assessments* and public policy as well as focused research to fill gaps in scientific knowledge." Over the last 20 years in general and the last 5 in particular, there have been numerous workshops, symposia, and other types of meetings designed to "set the stage for development of the next generation of methodologies for toxicological risk assessments" by examining how "*biomarkers* can significantly

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advance the understanding of some of the most critical elements of the *risk assessment process*" (Rodricks, 2000).

WHY USE BIOMARKERS IN RISK ASSESSMENT?

In the nearly two decades since Perera and Weinstein proposed four categories of biomarkers, there has been refinement and three distinct types of biomarkers have emerged. Biomarkers of exposure, effects of exposure, and host susceptibility parallel and are intimately linked to Schulte's (1989) amplification of the National Research Council (1987) exposure-disease continuum (Figure 1). The primary way that these three types of biomarkers may improve risk assessment is by reducing uncertainty. Biomarkers of exposure may reduce uncertainty associated with external dose measures in developing the dose-response analysis. For example, when extrapolating from the administered dose in an animal study, uncertainties due to interspecies differences in kinetics are reduced if the target tissue dose is known. In a quantitative dose-response assessment, this takes the form of using data to replace default uncertainty factors for the toxicokinetic portion of the factor for interspecies extrapolation. This approach is embodied in the use of chemical-specific adjustment factors (CSAF) to replace default uncertainty factors in risk assessment (IPCS, 2001). Exposure markers can also enhance the dose-response assessment by identifying measures most directly related to the response of interest, and therefore, increasing the specificity of the dose metric.

Effect biomarkers can reduce uncertainty in risk assessment in several ways. First, biomarkers of effect can increase the sensitivity of risk assessments,

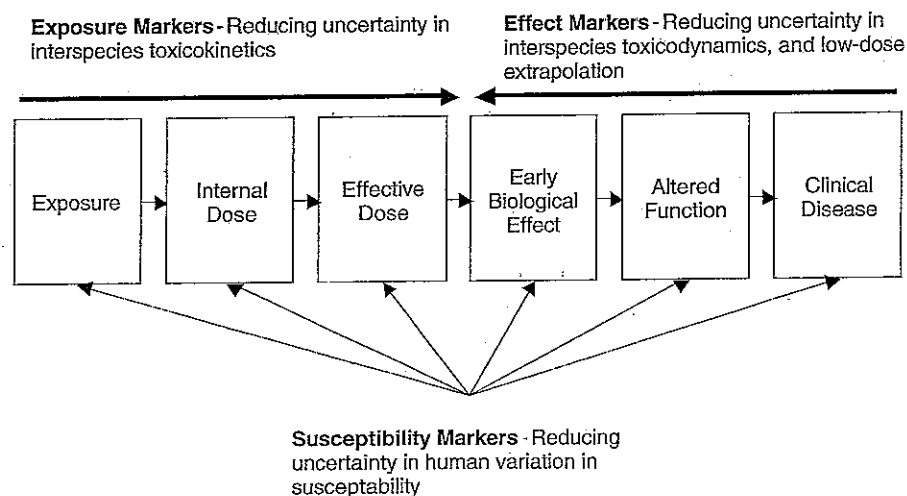


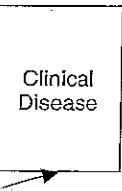
FIGURE 1. Schulte's (1989) amplification of the National Research Council (1987) exposure-disease continuum is shown. The impact of these biomarkers of exposure, effect, and susceptibility on uncertainties in risk assessment is presented.

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since such markers can often be observed at doses less than those that induce traditional default endpoints such as morbidity, mortality, tumors, body weight decreases, or tissue pathology. Current risk assessment practice is to use uncertainty factors to address extrapolation across levels of effect severity (e.g., from a lowest-observed-adverse-effect level [LOAEL] to a no-observed-adverse-effect level [NOAEL]), and therefore, identifying the appropriate effect metric can reduce the need for applying uncertainty factors to account for extrapolation from more severe effects. However, as noted in the discussion, there remains uncertainty in how sub-adverse effects can be used directly to identify a point of departure for risk assessment. Second, interspecies and intraspecies variability in the dose required to induce early effects can be used directly to derive CSAFs that replace default uncertainty factor values for toxicodynamics. Third, effect biomarkers reduce uncertainty in the choice of low-dose extrapolation models, where the underlying mechanism for induction of the early effect is known and presumptions about the likely shape of the dose-response curve can be made, or when the dose-response data for the effect biomarker can be used directly to assign a point of departure based on test concentrations that are immediately relevant to environmental exposure levels.

Biomarkers of susceptibility provide information on the human variability surrounding exposure or effect biomarkers, and provide additional opportunities to replace default uncertainty factors for human variability in risk assessments with factors based on data for the chemical of interest (e.g., CSAFs). The increasing use of CSAFs for human variability in kinetics and dynamics (IPCS, 2001) to replace default uncertainty factors provides a clear opportunity to make quantitative use of susceptibility markers.

STATUS OF IMPLEMENTATION

Since the early 1980s, biomarkers have gradually been incorporated and applied in varying degrees in distinct steps in the risk assessment process. However, one might ask if biomarkers have achieved their potential: Have they been utilized frequently as the basis for qualitative human health risk assessments, or have they gone the way of other, at-one-time highly touted assays with untapped potential that never quite realized an important and applicable niche in scientific research? The potential application of biomarkers in risk assessment has been recognized within the context of current risk assessment methods. For example, considerations for biomarker use have been described in U.S. Environmental Protection Agency (EPA) (1994) methods for deriving inhalation reference concentrations, in the context of mode of action assessments for cancer risk assessment (U.S. EPA, 1999), and in occupational toxicology as indicated by the development of biological exposure indices (ACGIH, 2001). Beyond that, the purpose of the current article is to determine the degree to which biomarker incorporation in the risk assessment process has occurred. Additionally, if the progress of biomarker implementation

into risk assessment is not satisfactory or has been impeded, the identity of barriers that have limited biomarker incorporation into the process is sought. Lastly, examples of how risk methods are evolving to incorporate biomarker information to address current barriers are presented

METHODS

In order to determine the extent of implementation of biomarkers into the risk assessment process, an unsophisticated, 4-question informal survey (Table 1) was developed and distributed to 30 leading risk assessment practitioners. Since the general goal was to examine the pulse of risk assessors, a deliberate effort was made to exclude biomarker experts from the survey. Within the survey, an attempt to address a number of specific issues was made. Principally, to insure that an appropriate group of experts was being surveyed, the respondents were asked to gauge their appreciation and understanding of biomarkers and how they might be employed in the risk assessment process. The second question dealt with whether or not the respondent could identify risk assessments where a specific type of biomarker (exposure, effects or susceptibility) had been successfully incorporated into the risk assessment. The third question asked the respondents to identify "barriers" that were impeding the effective and routine use of biomarkers into the risk assessment process. Lastly, respondents were asked to predict what technologies would result in biomarkers that would benefit the risk assessment process.

RESULTS

From the initial pool of 30, 13 risk assessors provided responses to the electronic mail survey.

Question 1—Level of appreciation (1 to 5; 5 highest) Nine of the respondents rated their understanding of biomarkers as 4 or greater. Two rated themselves at 3, leaving only two others at 2. In general, this indicated that the survey group felt knowledgeable/enlightened with regard to the subject matter.

Question 2A—Biomarkers of exposure in risk assessment Two chemicals, lead and mercury, were cited by nearly half of the respondents as examples where biomarkers of exposure were successfully and appropriately incorporated into risk assessments. Cadmium, ethanol, arsenic, dioxin, and formaldehyde all received multiple citations from the respondents.

Question 2B—Biomarkers of effects in risk assessment The respondents provided at least 10 examples where they believed that biomarkers of effects were successfully and appropriately incorporated into risk assessments. However, none of the cited examples received the majority of recognition that lead and mercury had garnered in question 2A.

Question 2C—Biomarkers of susceptibility in risk assessment From the pool of 13 respondents, only 5 potential biomarkers of susceptibility were submitted

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TABLE 1. Survey Questions and Question Responses With Frequency of Response

Question	Response (frequency of response)
Question 1: On a scale of 1-5 (5 being the highest), how would you rate your appreciation and understanding of biomarkers and their potential application to the risk assessment process?	1(0); 2(1); 3(2); 4(6) and 5(2) One respondent qualified his or her experience as "very good" while another described his or hers as "no experience."
Question 2A: Are you aware of successful risk assessments where Biomarkers of Exposure were effectively and appropriately employed? Please provide examples.	Lead (7); mercury (6); ethanol (3); arsenic (2); dioxin (2); formaldehyde (2); 4-aminobiphenyl (1); dimethylformamide (1); styrene (1); benzene (1); butadiene (1); acrylamide (1); aflatoxin (1); carbon monoxide (1); RBC cholinesterase inhibition (1); methylene chloride (1); and cotinine (1).
Question 2B: Are you aware of successful risk assessments where Biomarkers of Effects were effectively and appropriately employed? Please provide examples.	Cholinesterase inhibition by pesticides (3); pulmonary function by coal dust, silica (2); kidney effect markers, e.g., for cadmium (3); methemoglobin by NO (1); carboxyhemoglobin by methylene chloride (1); formaldehyde (1); thyroid hormones by perchlorate (1); allergic contact dermatitis by hexavalent chromium (1); DNA and/or protein adducts (3).
Question 2C: Are you aware of successful risk assessments where Biomarkers of Susceptibility were effectively and appropriately employed? Please provide examples.	Acetylator status (2); children cancer susceptibility (2); benzene (1); BRCA1 polymorphism (1); genes that predispose to copper effects (Menkes and Wilson's genes) (1).
Question 3: What do you see as the primary barrier to the effective and routine use of biomarkers in the risk assessment paradigm?	Lack of available data to identify biomarkers (4); lack of sufficient biomarker validation (4); nonspecific nature of many biomarkers (1); temporal dependence, predictivity of long-term disease (1); clearly developed paradigm on how to use subadverse biomarkers in risk assessment (1); premature use of biomarkers (1); resistance to use of biomarkers unless detailed mechanism of action known (1).
Question 4 (Extra Credit): What technologies do you foresee as resulting in Biomarkers that will prove useful to the risk assessment paradigm?	"Omics" (6); measurement technique/analysis (5); validations (2); PBPK modeling (1); currently unknown tests for susceptibility (1).

as having been successfully incorporated into a risk assessment. Two of these were cited twice—acetylator status and child cancer susceptibility.

Question 3—Barriers Two areas received strong support as central to the barrier question. These were the lack of available data to identify biomarkers and the lack of sufficient biomarker validation.

Question 4—The future Again, two areas were most frequently cited as crucial to the future development of biomarkers and their use in the risk assessment process. The first was loosely defined as the field of "omics." For the most part, this included proteomics and genomics, but metabonomics and other potential "omics" were also noted. The second technology cited as pivotal to the continued development of biomarkers for their use in risk assessments was improvements in sensitivity and specificity of potential biomarker measurements and analysis.

DISCUSSION

Survey Results

Although informal, unsophisticated, and perhaps nonscientific, the survey was designed to gauge the feeling of risk assessors on the value of biomarkers to their discipline. The first question was to ensure that the right people were being surveyed, while the second was designed to capture "quick thoughts" to highlight commonly established biomarkers and their use in risk assessment. The responses to the first question confirmed the belief that an appropriate sample had been identified. Interpreting the responses to the series of related questions in question 2 was more difficult.

Some of the biomarkers of exposure could be sorted into a "commonly recognized" group that many respondents were familiar with and accepted as useful. Mercury and lead biomarkers were cited and supported by nearly a majority of the respondents. A smaller set of chemical-specific biomarkers was identified by fewer respondents. This list could have been longer had the respondents been granted more time to reply or if more risk assessors had been surveyed (or if more of the 30 initially requested to participate had actually responded). It was concluded that for particularly well-studied compounds, acceptance of biomarkers of exposure for risk assessment purposes is high. A key point along those lines is that risk assessment methodology has been fully adapted to accommodate the incorporation of biomarkers of exposure. Other evidence for wide use of biomarkers of exposure is their employment in occupational applications such as the biological exposure indices (BEI) developed by the American Conference of Governmental Industrial Hygienists (ACGIH). For dose-response assessments, more and more risk assessments integrate physiologically based pharmacokinetic (PBPK) modeling for the estimation of internal and target tissue doses, rather than relying on administered dose as the appropriate dose-metric. Thus it appears that the primary limitation in using exposure biomarkers in risk assessment lies in the need for chemical-specific data.

Question 2B regarding biomarkers of effects elicited many potential examples, but few if any of the responses received high recognition from a majority. This suggested that while effects biomarkers are being used, their implementation is less universally accepted than exposure markers.

Another item of note from these data is that there appear to be difficulties in distinguishing a clear separation among some effective dose markers, early effect markers, and markers of adverse effects. For example, DNA adducts, carboxyhemoglobin (COHb), and red blood cell (RBC) cholinesterase inhibition were selected by some respondents as exposure markers, while others classified them as effect markers. Additionally, pulmonary function test (PFT) changes were considered markers of effect by some, but would be considered as clear adverse effects by others. These results suggested that early effect markers are gaining use in risk assessment, but that methods have not sufficiently evolved to address a critical underlying issue—how to incorporate sub-adverse effects in the dose response in the absence of a validated biologically based dose-response (BBDR) model.

The dearth of responses suggesting examples of biomarkers of susceptibility indicates that these markers are the least well understood or the most difficult to incorporate into the risk assessment process and have the most questions to address before they can gain wide acceptance for routine use by the risk assessment community.

Breaking Down the Barriers

The survey results suggest that while exposure markers are well on their way to full integration in risk assessment, maturation of risk methods is needed to capitalize on biomarkers of effect and susceptibility. Recent developments in risk assessment approaches highlight how this maturation is taking place. Two examples are noted here.

First, methods are being employed to facilitate the quantitative use of early effect markers in risk assessment. BBDR models represent a thorough integration of mechanistic understanding into the risk assessment process, but are resource intensive and therefore limited to few chemicals. In the nearer term, the use of categorical regression methods to incorporate biomarker data in the dose-response assessment will be explored. The strength of the categorical regression approach lies in its ability to integrate response data for effects of differing severity to develop an overall cumulative probability relationship (Haber et al., 2001). Because this methodology weds low-dose and high-dose responses, it provides a potential novel method for validating early effect biomarkers, by identifying inconsistencies in the dose-response curves for low-dose and high-dose effects. This tool has been used to include early effect data in risk assessments for cholinesterase-inhibiting pesticides (Dourson et al., 1997; Teuschler et al., 1999).

Second, approaches for quantitative use of genetic polymorphism data are being demonstrated. For example, as shown in Figure 2 for warfarin, information on a genetic polymorphism was combined with PBPK modeling and

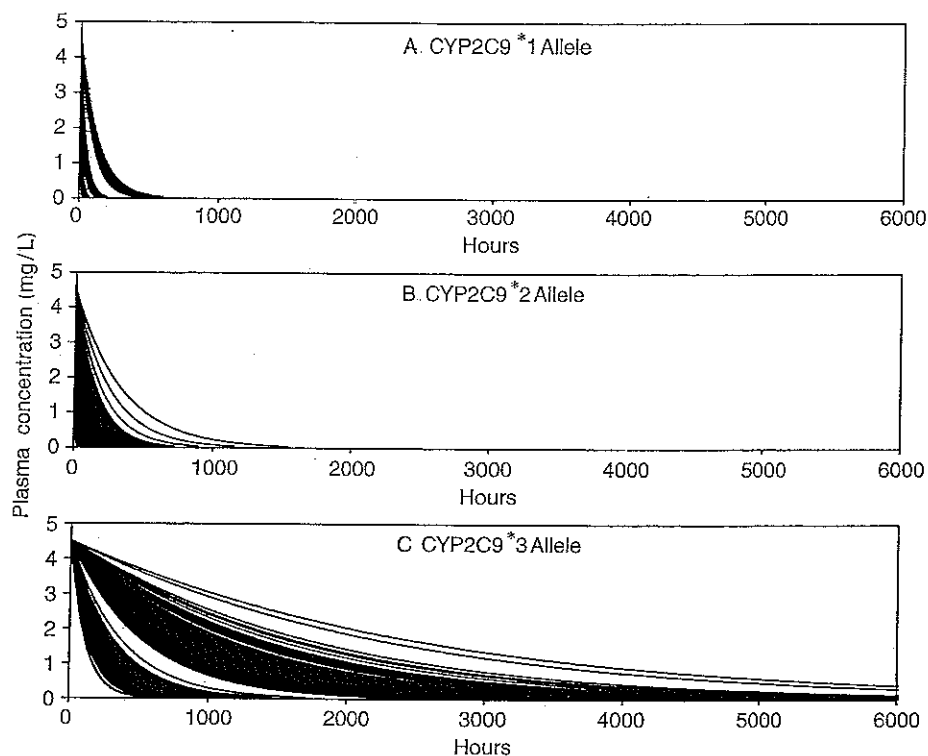


FIGURE 2 The effect of metabolism by the three alleles of CYP2C9 on the concentration of (S)-warfarin in human plasma is shown (from Gentry et al., 2002). The observed effect of genetic polymorphisms on variability in target tissue dose can be used quantitatively to replace default uncertainty factors for human variability in toxicokinetics.

information on physiological variability to estimate variability in target tissue dose (Haber et al., 2002; Gentry et al., 2002). The resulting approach can be used directly in the risk assessment process for assigning a CSAF to account for human toxicokinetic variability.

CONCLUSIONS

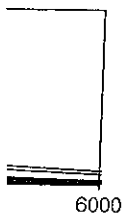
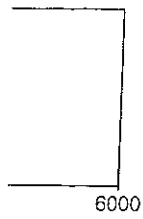
Based on the informal survey and nonscientific analysis of the responses, the following thoughts relevant to the incorporation of biomarkers into the risk assessment process emerged:

- Biomarkers are increasingly being applied to risk assessments, with exposure markers being routinely used, effect markers seeing variable application, and susceptibility markers needing the greatest degree of development.

- Data from new technologies will increase biomarker use in risk assessments. Survey responders specifically noted the potential of toxicogenomics as a technology that will impact on biomarker identification.
- Risk methods are evolving to incorporate biomarker information. For example, the common use of PBPK modeling and the CSAF approach has cemented a place for exposure markers in risk assessment. Furthermore, examples were discussed on how the methods are evolving to increase the use of effect and susceptibility markers

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