The Role of Specimen Banking in Risk Assessment

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The risk assessment process is described with a focus on the hazard identification and dose–response components. Many of the scientific questions and uncertainties associated with these components are discussed, and the role for biomarkers and specimen banking in supporting these activities are assessed. Under hazard identification, the use of biomarkers in defining and predicting biologically adverse events; 

Background

As citizens, we are all concerned that our health, and the health of our children could be compromised or endangered by exposure to toxic chemicals and other potential health hazards in the air we breathe, and in our food and drinking water. Public concern over this potential for harm resulting from exposure to environmental pollutants has led to a demand for protection from environmental risk, either real or imagined. This demand has prompted public health officials, environmental scientists, and regulatory agencies to pursue processes to define, explain, and mediate environmentally related health risks (1,2). The U.S. Environmental Protection Agency (U.S. EPA) has responded to public demand by adopting a paradigm that was proposed initially by the National Academy of Sciences (NAS) (3). This approach to risk assessment provides a format and data for estimating the potential adverse health effects of human exposures to environmental hazards that, in turn, provides the cornerstone to risk management decisions (Figure 1). In this article, selective components of this risk assessment process are described as well as some of the scientific questions and uncertainties that accompany these components. The role for biomarkers and specimen banking in this process will be assessed. The discussion focuses on issues related to human exposure and health assessment rather than the broader role of biomarkers in toxicology (e.g., mechanistic studies in animals). Although much has been written on the role of biomarkers to risk assessment, the challenge to participants in this symposium is to define the potential contributions to be gained from specimen banking activities.

Although this paper is focused on risk assessment, it should be recognized that information required for this process is also critical for numerous other collateral and interrelated activities and actions that support risk management decisions. Biomarkers and specimen banking may also contribute to these activities, and, in fact, their role may be even more apparent in these other contexts. For example, information derived from activities that may be referred to as monitoring (exposures) or surveillance (health status and trends) are essential to establishing baseline (reference) values, directing pollution prevention options, assessing the efficacy of corrective actions, or anticipating/detecting emerging environmental problems. Such data when combined with risk assessment activities may also help define and prioritize the legitimate environmental risks for the public. This approach can, in turn, ensure that public and private attention, expertise, and resources are directed appropriately. An appreciation of the interplay between these collateral activities and risk assessment should be incorporated into defining the role and criteria for biomarkers and specimen banking activities purported to support these processes.

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Figure 1. Components of the risk assessment process and the interface with risk management.

For this paper, biomarker will be defined "as any measurable biochemical, physiological, cytological, morphological, or other biological parameter obtainable from human tissues, fluid, or expired gases, that is associated (directly or indirectly) with exposure to an environmental pollutant" (4).

The Risk Assessment Process

Simply defined, risk assessment is the attempt to understand the relationship between human exposures and potential health effects. This understanding requires identifying the factors that result in human exposure and then defining the cascade of events that must occur to create a health risk. This analysis entails delineating the pharmacokinetic and pharmacodynamic processes that govern this cascade. As presented in Figure 2, biomarkers research has separated historically this continuum into exposure and health compartments. More contemporary efforts have acknowledged that biomarkers of dose can serve as the common denominator for linking these
events. Future efforts should approach understanding these events from a continuum perspective.

The NAS risk assessment paradigm defines four components that can be overlaid on this continuum (Figure 3):

**Hazard Identification.** Does the agent cause an adverse effect?

**Dose–Response Assessment.** What is the relationship between dose and occurrence/magnitude of health effects in humans?

**Exposure Assessment.** What exposures are experienced currently or anticipated under different conditions?

**Risk Characterization.** What is the estimated occurrence/magnitude of the [adverse] effect in a given population?

Conventionally (and conveniently) biomarkers can be defined by three, interrelated categories, namely, biomarkers of exposure, effect, and susceptibility that can be related to the components of the risk assessment process. These relationships are discussed in the following sections. Since much of this symposium is devoted to human exposure, the focus of this paper will be primarily on biomarkers as they relate to hazard identification and dose–response assessment.

**Biomarkers and Hazard Identification**

Hazard identification defines whether an agent can cause an adverse effect and its relevance to human health and disease. This evaluation examines all available data including human, test species, and *in vitro* data with close scrutiny to dose–response and dose–effect relationships. Conclusions as to the hazard potential for a given agent are based upon a weight-of-evidence summation.

There are several issues that must be addressed in hazard identification in which human biomarker data could contribute (Table 1). Perhaps most critical is understanding the biologic significance of biomarker(s) of effect whose occurrence can be measured at very low exposure levels. Because of increasing evolution and sophistication in measurement methods and instrumentation, changes in baseline levels can be detected more readily. Given an appropriate study design, these changes may be found to be statistically significant. Less certain is whether these biomarkers represent an adverse, or potentially adverse event, i.e., their value in predicting human health disease or dysfunction. (For the remainder of this paper, “disease” will be used to imply either a dysfunctional state or actual disease.) An example of a success story is blood lead levels in children and the demonstrated relationship to neurotoxicity that has provided key information for the existing lead standard. On the other hand, the biologic significance of moderate changes in plasma and red blood cell levels of acetylcholinesterase (AchE) remains uncertain. Although widely accepted as a biomarker of exposure to certain classes of pesticides, the role of peripheral levels of AchE in predicting toxicity to the central nervous system (CNS) is less clear.

Ideally, biomarker(s) of effect should provide insight into current health status and, if present, the stage of the disease. An understanding of the potential for reversibility associated with a decrease or discontinuation of exposure is equally important. Biomarkers of reversibility however, must distinguish between true recovery (absence of pollutant-induced effects) and the failure to detect adverse effects as a result of adaptation or biologic compensation which may mask existing impairment. There is also a need to understand the relationship between the current biomarker and silent processes that may underlie the [eventual] appearance of disease. The common denominator for a biomarker of effect that provides information on the latency, stage and progression, and reversibility is an understanding of the putative mechanisms of the disease under study.

Potentially, biomarkers may also play a role in determining if a threshold exists, and, if so, what level of exposure is necessary to exceed that threshold and pose a health risk. Equally important is whether this threshold varies for different populations (e.g., young vs adult, rural vs urban). Biomarkers that can identify/distinguish these populations may impact dramatically health risk assessment and risk management decisions.

**Biomarkers and Dose–Response Assessment**

Critical to any risk assessment is an understanding between exposure (dose) and the occurrence/magnitude of adverse effects. Ideally, this relationship would be approximately linear (i.e., increasing risk with increasing exposure). However, depending on the target and its inherent properties to respond to toxicity (e.g., repair), a matrix of exposure and effects scenarios is more likely (Table 2). The situation becomes more complicated when an individual operates concurrently under more than one exposure situation (e.g., chronic, low-level exposure with periodic high excursions) and experiences multi-chemical exposures. The use of biomarkers of dose and pharmacokinetic modeling offers great promise for better defining exposure (dose)–response relationships.

Henderson et al. (5) have proposed that a suite of biomarkers be employed to reflect recent as well as past and, potentially, cumulative exposures. Such a suite would accommodate varying rates of disposition (e.g., different half-lives) of the parent compound, its metabolites, and any other surrogate markers that reflect an interaction between the agent and a biologic target (Figure 4). Yet, as seen in Figure 5, even an accurate estimate of dose may not predict effect status. Again, understanding of the pharmacokinetic behavior of an agent must be synthesized with
hypotheses/insights into the processes and mechanisms of the disease in question to provide biologically plausible dose–response assessments.

Although such biologically based models are desirable, such approaches, to date, have had limited application, primarily focused on cancer and favoring a no threshold hypothesis (i.e., the interaction of a single molecule in a single cell will result in an adverse effect). The prospect of nongenotoxic, carcinogenic mechanisms has suggested that thresholds may, in fact, exist for certain environmental carcinogens.

A threshold is assumed to exist for most noncancer health effects. That is, there is a range of exposures from zero to some finite level that can be tolerated with essentially no adverse effect. These assessments most often rely on defining a no observable adverse effect level (NOAEL) or a lowest observable adverse effect level (LOAEL) from the available data. This estimate is then adjusted downward by application of a series of uncertainty factors to provide a conservative estimate of an exposure (dose) level which poses no human health risk. This process of defining a LOAEL or NOAEL and applying uncertainty factors (Table 3) produces what is now widely termed a reference dose (RfD) or reference concentration (RfC) depending on whether the exposure route is oral or inhalation, respectively. For purposes of this paper, discussion will focus on the uncertainties associated with high-to-low dose extrapolation and the across human (interindividual) variability. Irrespective of whether biologic modeling or an RfD/RfC approach is employed, uncertainties associated with these two factors will be present. Biomarkers may have a substantial role in determining the necessity and/or magnitude of these uncertainty factors.

High-to-Low Dose Extrapolation

Although human health data for the exposure situation of concern is what is desired, the majority of data on which human risk assessments are based is derived from test species or humans in elevated exposure settings (e.g., occupational). The risk assessor is then required to determine risk for individuals operating in environments characterized by much lower exposures. The shape and slope of the curve of a dose–response model or the nature and magnitude of uncertainty factors applied to a NOAEL are related directly to the confidence that the targets and mechanisms underlying the toxic response in the test species are comparable in humans and that those mechanisms can be triggered at the lower doses associated with environmental exposures.

The tendency historically has been to accept these assumptions with research then focused on identifying biomarkers of mechanism and dose present at low exposure levels. This approach assumes implicitly a linear relationship between dose and risk. Perhaps a more systematic approach would be to identify biomarkers nearer the dose range of the experimental data and then progress in a descending, stepwise fashion toward the human exposure range of concern. Thus, initial efforts would compare biomarkers from humans with exposures closest to the experimental data (Figure 6). Such data would most often come from the occupational setting.

High-to-low dose extrapolations may assume initially that the highest exposed individuals are biologically representative of the general population and differ only in terms of exposure. Clearly, other factors pose limitations to this overall generalization. For example, the healthy worker effect in occupational settings may produce exposure–response data that underestimate health risk for the general population even at lower exposures. Conversely, if the highest exposed also represents groups with compromised health status (e.g., the poor, the elderly), extrapolation may overestimate the effect(s) for the general population.

Interindividual Variability

The examples presented above may be considered to be a subset of the many factors associated with interindividual variability in response (i.e., biomarker) in a given environmental setting. Other terms often used

Figure 4. Potential, relative levels of various biomarkers with time after exposure (5).

Figure 5. Varied time–effects scenarios even with the concentration of an agent at steady state (heavy dark line).

Table 3. Uncertainty factors generally considered in developing an RfD/RfC.

| Tenfold | Within human variability       |
|---------|--------------------------------|
| Tenfold | Animal to human variability    |
| Tenfold | Subchronic to chronic exposure |
| Tenfold | LOAEL to NOAEL                 |
| <1 – tenfold | Modifying factor for other uncertainties |

Figure 6. Overlay of possible human exposure distribution on the extrapolated dose-risk curve (dashed line). The band demarcated by vertical lines represents a human subgroup with the highest exposures. The question mark reflects uncertainty as to the shape of the actual curve below the observed data.
to account for interindividual variability are differences in sensitivity or susceptibility of the individual or subpopulation to a specific environmental insult. Whether these phenomena, in fact, reflect the same, underlying biologic processes is debatable and certainly has ramifications for interpretation of biomarker data. However, this question could serve as the basis for the entire symposium and will not be addressed in this paper.

The major premise is that, although individuals may experience similar environmental exposures, individual differences in pharmacokinetic or pharmacodynamic processes may greatly influence the dose that reaches the target site and/or the degree of response. A number of factors including age, diet, and health status will obviously influence these processes. Increased or decreased responsiveness (susceptibility) may also be acquired wherein previous exposures sensitize the individual to subsequent exposures. An immunologic basis is likely for this phenomena.

However, genetic predisposition seems to be the major determinant. For example, inherited differences in metabolic capabilities (e.g., polymorphism for activating/deactivating enzymes) can greatly influence the concentration and maintenance of the biologically effective dose at the target site. Similarly, genetic differences in repair or compensatory mechanisms, reserve capacity, and other biologic processes may influence the magnitude of the toxic response.

The existence of interindividual variability in response implies that the individuals at greatest health risk may not be synonymous with those that experience the greatest exposures. The interplay between these two distributions is not well understood (Figure 7). Biomarkers that provide such insights will greatly assist efforts to quantify human risk estimations.

The Role of Tissue Banking

Based upon the preceding discussion, biomarkers of exposure, effect, and susceptibility would appear to have major roles in improving risk assessments. How the retention and preservation of these samples (specimen banking) may further enhance these estimations is less clear. Moreover, the application will usually be retrospective, i.e., banking specimens today that may improve, refine, or reaffirm a risk assessment addressed in the future. Such an application places a tremendous burden on the population sampling design for a specimen bank since it is critical that individuals/groups sampled today be representative of the exposed population in which disease is observed in the future. Some potential, interrelated applications can be offered that have implications for hazard identification and dose–response assessment.

a) Reaffirm biologic significance/predictive validity: This application requires retrospective comparisons, namely, determining the relationship between previously obtained biomarker(s) and current exposure/health status. The ability of specific biomarkers to predict disease progression and reversibility may also be ascertained. Such evaluations would allow greater confidence to be placed on preclinical, low dose biomarkers as the basis for a risk assessment in the absence of frank disease.

b) Provide historical baseline (reference) values: The ability to ascertain whether an agent has elevated health risk can be strengthened by comparison to concurrent control values which have been placed in the context of historical values. This comparison of control cohorts may allow for the discrimination and quantification of temporal versus pollutant-induced changes in a given health measure.

c) Reassess mechanistic hypotheses: As noted previously in this paper, identifying and understanding pharmacokinetic and pharmacodynamic mechanisms are key to ensuring more biologically sound risk assessments. Specimen banking may allow the retrospective testing of hypotheses regarding putative mechanisms for diseases, especially those with long latencies. Again, the current and future cohorts must be similar enough to allow such linkages to be valid. This application is facilitated if the specimens were obtained on the actual target tissues (e.g., lung, liver, etc.), or if concentrations in biologic fluids have been demonstrated to truly reflect target dose.

d) Confirming exposure–dose effects linkages under changing exposure scenarios: As exposure conditions of a population or sub-group change over time a corresponding change in health status (i.e., biomarker values) should occur if previously hypothesized associations and attendant risk estimations are valid.

e) Identifying new high risk groups: Factors that may elevate the risk to an environmental pollutant for certain individuals or subgroups (e.g., increased exposures; increased susceptibility) will impact the risk assessment for that agent. Banked specimens may provide the referents to aid such identification.

Conclusions

The concept of tissue banking is to provide for the long-term storage of biologic specimens. The premise is that a bank of tissue samples, collected and archived appropriately, provides scientifically preserved and documented samples for retrospective and prospective cohort studies. This resource, by providing human material, would also seem to hold great promise for reducing many of the uncertainties associated with assessing the health risks associated with exposure to environmental pollutants.

The design and implementation of a specimen bank should benefit from participation of diverse areas within the scientific community (e.g., epidemiologists, toxicologists, industrial hygienists, statisticians, risk assessors, etc.). This broad input is critical to determine whether a design for specimen banking can be developed that will accommodate divergent interests/needs within the public health community. To that extent, the compatibility of risk assessment needs relative to other applications will require further exploration.

REFERENCES

1. Allman WF. We have nothing to fear (but a few zillion things). Science 85:38–41(1985).
2. Bender AP, Williams AN, Johnson RA, Jagger HG. Appropriate public health responses to clusters: the art of being responsibly responsible. Am J Epidemiol 132:548–552(1990).
3. National Research Council. Risk Assessment in the Federal Government: Managing the Process. Washington:National Academy Press, 1983.
4. Griffith J, Duncan RC, Hulka BS. Biochemical and biological markers: Implications for epidemiologic studies. Arch Environ Health 44:375–381(1989).
5. Henderson RF, Bechtold WD, Bond JA, Sun JD. The use of biological markers in toxicology. Crit Rev Toxicol 20:65–82(1989).