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Biomarkers, Bioindicators, and Ecological Risk Assessment—A Brief Review and Evaluation

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Cellular and subcellular measures of exposure (biomarkers) and effects (bioindicators) continue to be developed. This development is justified in part by the potential application of these technologies in supporting ecological risk assessment (ERA). However, application of biomarkers in assessing ecological risk remains infrequent and of questionable utility. The following discussion examines the potential for increasing the utility of biomarkers and bioindicators in ERA. Recent studies suggest that biomarkers can contribute to most aspects of a commonly used framework for ERA. Methods that address gene expression (i.e., proteomics) appear particularly promising in terms of economy in application and significance of results. The primary challenges in using biomarkers/bioindicators to assess risk include the difficulties in (1) developing stressor-specific, quantitative dose-response functions and (2) projecting higher-order ecological effects from cellular or subcellular bioindicators.

Keywords biomarkers, bioindicators, risk assessment

Biomarkers provide at least two key attributes in environmental toxicology (Ricketts et al. 2003). First, biomarkers are, by definition, responsive only to the biologically active fraction of accumulated body burden of one or more toxicants. That is, biomarkers characterize the bioavailable fraction of environmental chemicals. Second, biomarkers integrate the interactive effects of complex mixtures of chemicals experienced by organisms in ecosystems impacted by modern industrial and agricultural chemicals.Biomarkers offer one possible solution to the recognized limitations of extrapolating the results of single-chemical, single-species laboratory toxicity assays in assessing ecological risks. Therefore, it is not surprising that one justification of the continued development, application, and evaluation of biomarkers (and bioindicators) is based on their potential contribution to ecological risk assessment.

Discussion of the potential contribution of biomarkers and bioindicators to the practice of ecological risk assessment (ERA) would benefit from an operational definition of these terms. The term *biomarkers* has been defined as any functional measure of exposure that is characterized at a suborganism level of biological organizations (Adams et al. 2001). Biomarkers can be measured at molecular, biochemical, cellular, or physiological levels of biological organization (Ricketts et al. 2003). The World Health Organization defines a biomarker as "any substance, structure, or process that can be measured in the body…and influence or predict the incidence or outcome of disease." Biomarkers can be classified as markers of exposure, effects, and susceptibility (WHO 2001).

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In contrast, *bioindicators* has been used to identify structures or processes indicating exposure or effects measured at higher levels of organization (e.g., organism, population, community, ecosystem) (Adams et al. 2001). Note, however, that Adams (2003) uses the term bioindicator to refer to biological responses expressed from the biomolecular-biochemical levels to population and community levels of organization. Burger and Gochfeld (2001) identified three general characteristics that should be considered in selecting bioindicators: biological relevance, methodological relevance, and societal relevance. Table 1 lists several more specific attributes of these broader characteristics. These authors maintained that failing to consider these aspects would likely result in the eventual abandonment of bioindicators otherwise selected.

McCarty and Munkittrick (1996) attempted to integrate the discussion of biomarkers and bioindicators by broadly defining bioindicators to include biochemical, physiological, or ecological structures or processes that have been correlated or causally linked to biological effects measured at one or more levels of biological organization. Following this broader definition, Table 2 provides several examples of biomarkers and bioindicators for different levels of organization. Each of the six selected levels represents an increase in level of organization, an associated decrease in sensitivity of response to environmental stressors, and corresponding increase in ecological relevance (Adams and Greeley 2000). For the purpose of this essay, *biomarkers* and *bioindicators* will be used somewhat interchangeably, with *biomarkers* used more to emphasize measures of exposure or dose and *bioindicators* referring more to measures of effects.

The following discussion focuses on those desirable attributes of biomarkers and bioindicators that would increase their usefulness in assessing ecological risks. A comprehensive review (e.g., Moore et al. 2004) of technical progress in the continuing development and application of biomarkers and bioindicators for assessing risk lies beyond the scope of this presentation. Selected studies will be briefly described to reinforce the importance of these attributes and to provide point-counterpoint examples of the strengths and limitations of biomarkers and bioindicators in estimating ecological risk. The potential

Table 1
Desired characteristics of bioindicators for assessing ecological well-being (adapted from Burger and Gochfeld 2001)

Biological relevance
Low natural variability
Measurable changes in response that are attributable to specific stressor
Responses that persist and that are ecologically significant
Responses that encompass variations in scale and complexity
Methodological relevance
Economically and accurately monitored
Conveniently measured over the long term
Specified methods for data collection, straightforward analysis, and unambiguous Interpretation
Supports hypothesis testing
Societal relevance
Responses deemed important by society
Responses easily understood and scientifically defensible
Responses that are cost effective

Table 2
Example biomarkers and bioindicators measured at different levels of biological or ecological organization (adapted from Adams and Greeley 2000)

Biochemical	Physiological	Histopathological	Individual	Population	Community
MFO enzymes	Creatinine	Necrosis	Growth	Abundance	Species richness
Bile metabolites	Transaminase enzymes	Macrophage aggregates	Total body lipid	Size and age distribution	Index of biotic integrity
DNA integrity	Cortisol	Parasitic lesions	Organo-indices	Sex ratio	Intolerant species
Stress proteins	Triglycerides	Functional parenchyma	Condition factor	Bioenergetics	Feeding guilds
Antioxidant enzymes	Steroid hormones	Carcinomas	Gross anomalies	Reproductive integrity	

contribution of biomarkers and bioindicators to ecological risk assessment will be examined in the context of a recognized approach for assessing risk.

There are several conceptual frameworks that guide ecological risk assessment internationally (Bartell 1996). However, the USEPA (1998) methodology will be used to structure the following discussion because of the widespread familiarity and implementation of this approach among practicing risk assessors and decision makers. Following this discussion, several consensus recommendations are suggested for increasing the contribution of biomarkers and bioindicators in assessing ecological risk.

Schulte and Waters (1999) previously explored a general rationale for incorporating biomarkers into an improved approach for risk assessment (Figure 1). More traditional risk assessments emphasize the derivation of empirical or process-oriented relationships between some measure of exposure external to the organism and a corresponding lethal or sublethal response of interest. In contrast, biomarkers offer the promise of more specific and internal characterization of a biologically effective dose. An internal indicator of exposure to a stressor could advance the practice of risk assessment by removing some of the assumptions and uncertainties inherent in evaluating bioavailability and estimating a meaningful dose from concentrations of chemical contaminants measured in the environment (e.g., air, soil, water, and sediment). Similarly, biomarkers of effects could improve risk assessments by providing highly specific internal measures of detailed biological response at cellular or subcellular locations targeted by contaminants. Biomarkers could, in theory, provide information concerning specific modes of action and associated toxicological responses observed subsequently at successively higher levels of biological organization. Taken together, biomarkers of exposure and biomarkers (bioindicators) of response could then be more meaningfully integrated and thereby advance the practice of ecological risk assessment.

Using the USEPA framework, the following sections outline how biomarkers and bioindicators might contribute to the performance of *problem formulation*, *analysis of exposure*, *analysis of effects*, and *risk characterization* – the main components of an ecological risk assessment performed in accordance with USEPA (1998).

Problem Formulation

A key objective of problem formulation is the construction of a conceptual model that comprehensively outlines the intended risk assessment (USEPA 1998). A carefully designed conceptual model can set the stage for an effective and useful ecological risk assessment. One of the major challenges in usefully incorporating biomarkers and bioindicators into

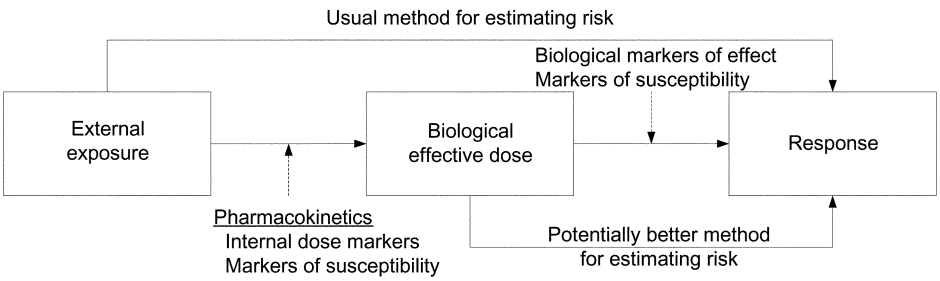

Figure 1. Rationale for using biomarkers to assess risk (adapted from Schulte and Waters 1999).

ERA lies in the design of well-articulated conceptual models that include exposure analyses, analysis of effects, and risk characterizations that span levels of organization from biochemistry to ecosystems.

For biomarkers to enter more frequently into risk assessment, the development of conceptual models will need to increasingly reflect consideration and meaningful inclusion of available and appropriate markers. Such consideration might be encouraged through more convenient and accessible communication of applicable biomarkers to more traditionally trained environmental toxicologists, who might otherwise focus on more conventional acute and chronic toxicity assays in structuring conceptual models for ecological risk assessment. An interactive, user-friendly decision support system should be constructed to assist risk assessors in the intelligent selection and incorporation of biomarkers and bioindicators into conceptual models for ecological risk assessment. Desirable features of such a support system would include (1) the ability to identify specific biomarkers in relation to chemical stressors of suspected importance in an assessment; (2) specification of process-level relationships between selected biomarkers and bioindicators of effects, especially effects measured at the whole-organism level (e.g., behavior, survival, growth, reproduction); (3) characterization of population- or community-level implications of the whole-organism(s) effects; and (4) estimation of the benefit-cost ratio for using the biomarkers instead of more traditional approaches based on conventional environmental toxicology.

Following the development of a conceptual model that usefully included biomarkers and bioindicators, a corresponding work plan would describe a detailed program for sample collection and analysis of selected biomarkers and bioindicators. The analysis plan would address the anticipated variability in biomarker measurements in relation to the number and size (age) of sampled organisms, and also would consider other environmental factors that could influence such measurements.

Analysis of Exposure

The early development of methods for assessing ecological risks relied upon existing toxicity assays that were designed to relate concentrations of chemicals in environmental media to associated lethal (e.g., LC_{50}) or sublethal (e.g., reproductive impairment) ecological effects (Suter 1993). Analysis of exposure in ERA correspondingly focused on quantifying environmental concentrations of chemical contaminants and did not address the actual accumulation of those chemicals by exposed organisms. Exposure, thus defined, supplanted the concept of dose in ecological risk assessment. There appear to be at least two reasons underlying this approach to exposure analysis: one, it is impractical (uneconomical), or in many cases impossible, to acquire detailed measures of dose for most combinations of chemicals and organisms; and two, the regulation of chemicals from an ecological perspective remains based on more readily obtained concentrations in the environment. The current ERA methodology relies upon external exposure concentrations as a correlate for more detailed measures of bioaccumulation and dose.

Biomarkers can help improve the analysis of exposure if their measurement (1) indicates unequivocally that exposure to the stressor(s) of concern has occurred, (2) permits identification of specific stressors or combinations of stressors, and (3) provides a quantitative estimate of exposure (i.e., magnitude, duration). An effective biomarker of exposure would take the form of an economical and reliable biochemical or physiological measure that demonstrates bioaccumulation of a specific chemical or class of chemicals. A useful biomarker of exposure would provide direct evidence of chronic accumulation of toxic

chemicals that are readily metabolized (e.g., PAHs) and where parent compounds or degradation products are difficult or prohibitively expensive to routinely measure. Encouragingly, continued research and development of biomarkers has produced increasingly specific indicators of exposure and dose. For example, Ogunseitan (2000) lists more than 20 inducible enzymes and stress proteins produced by microbes in relation to different environmental stressors (Table 3). The important contributions to assessing risk appear as responses of specific proteins related to different specific stressors. As indicated in Table 3, microbial biomarkers have been developed for chemical (e.g., mercury, nutrients, PAHs) and non-chemical (e.g., UV radiation, virus infection) stressors of current regulatory concern. However, the limited number of quantitative relationships between measured biomarkers and the magnitude of exposure (i.e., dose) could impede the use of biomarkers to quantify exposure in assessing ecological risk.

Table 3
Microbial proteins that indicate ecosystem health (adapted from Ogunseitan 2000)

Ecosystem condition	Organism	Protein
	Inducible enzymes	
Mercury pollution	*Pseudomonas spp.*	Mercuric reductase, organomercurial lyase
PAH pollution	*Pseudomonas spp.*	Naphthalene dioxygenase
Chlorinated aliphatic hydrocarbons	*Methanococcus spp.*	Methane monooxygenase
Methane production	*Methanobacterium* and *methanosarcina*	Coenzyme F420 proteins
Nitrogen cycling	*Pseudomonas spp.* and *cyanobacteria*	Nitrate reductase and nitrogenase
Phosphorus cycling	*Alcaligenes eutrophus* and *cyanobacteria*	Polyphosphate kinase and phosphatase
Carbon cycling	*Phanerochaete chrysosporium* and *phytoplankton*	Lignin peroxidase, cellulase, ribulose 1,5-bisphosphate carboxylase
	Stress proteins	
Hypersalinity	*Bacillus subtilis*	GroEL, DnaK, ClpP
Nutrient depletion	*Vibrio spp.* *Bjerkandera spp.* *Pycnoporus cinnabarinus*	30 kDa protein peroxidase phenol oxidase
Hypothermal shift	*Halobacterium spp.* *Escherichia coli*	Hsp 21–28, 75–105 GroE, DnaK
Oxygen tension	*Bacteroides fragilis*	Hsp 60, 90, 106
Ultraviolet light irradiation	*Phormidium laminosum*	Hsp 33, 86, 89
Virus infection	*E. coli*	DnaK, GrpE, UspA
Toxic chemical pollution	*E. coli* and *photobacterium*	Proteins associated with loss of bioluminescence
Irradiation	*B. fragilis*	Hsp 95, 100

The continuing progress in the development of proteomics may provide a biomarker-based technology for exposure analysis that meets many of the above stated requirements (Bradley et al. 2002). This technology examines changes in the protein expression of hundreds to thousands of genes by organisms exposed to single (e.g., Shepard and Bradley 2000) or multiple (Shepard et al. 2000) stressors. Measures of protein expression signatures (PES) are becoming increasingly efficient (and economical) as the result of increasingly automated methods and computer programs that can analyze patterns of proteins produced by two-dimensional gel electrophoresis. In the near future, computerized chips containing thousands of oligonucleotide fragments might permit diagnosis of multiple stressors on the basis of key proteins (Potyrailo et al. 1998).

Analysis of Ecological Effects

Bioindicators have been developed as measures of ecological effects and are becoming increasingly commonplace in ecological risk assessment (Lorenz 2003). Ecological responses to stressors can be measured at many different levels of biological (e.g., biochemical, subcellular, cellular, individual organisms) and ecological (e.g., populations, community, ecosystems, landscapes) organization. This diverse set of effects can be a source of confusion in terminology if a biochemical or physiological effect is selected as a bioindicator. Investigators have differentiated biomarkers of exposure and biomarkers of effects for biochemical or physiological measures (Suter 1993). One advantage of biological indicators of effects lies in developing and understanding relationships between exposures measured using biomarkers and responses measured at correspondingly detailed levels of organization. Reliable relationships of this kind would permit the establishment of early-warning or "sentinel" measures of exposure and anticipated ecological effects. The continued development and accumulation of reliable sentinel biomarkers and bioindicators could augment current capabilities in assessing ecological risk.

Nevertheless, one of the drawbacks in using effects measured at cellular and subcellular levels of organization as bioindicators in ecological risk assessment is the perception that such effects are ecologically difficult to interpret or that the ecological significance is not known or readily estimated. The regulatory climate reflects a long-practiced emphasis on undesired impacts defined and measured at individual, population, community, and ecosystem levels of organization. Such emphasis might simply stem from the dominant role of whole organism toxicity data in the historical development of ecological risk assessment concepts (and methods) combined with the initial slow development and application of cellular and subcellular measures. Regardless, the future contribution of biomarkers and correspondingly scaled bioindicators to risk assessment will undoubtedly be determined by the ability to extrapolate such highly resolved biochemical measures to effects on (at least) whole organisms.

Central to the practice of ecological risk assessment and particularly to the analysis or characterization of ecological effects is the development of a quantitative relationship between exposure (dose) and an ecological response of interest to managers and decision makers (Bartell 1996; Bartell et al. 1992; Suter 1993). To contribute meaningfully to ERA, it is paramount that exposure-response functions be derivable (and derived) for biomarkers advocated to support risk assessment. Towards this end, the field of proteomics promises to generate PES that can be used to develop quantitative relationships between the degree of exposure and subsequent dose (Shepard and Bradley 2000).

Risk Characterization

Examination of exposure (dose) in the context of exposure-response functions provides an initial characterization of risk. One of the main challenges in realizing the potential power of biomarkers in characterizing ecological risk assessment has been the relative inability to mechanistically and reliably relate biomarkers to relevant higher-order ecological effects (McCarty and Munkittrick 1996; Adams 2003). The following studies were selected to illustrate some of the strengths and limitations in developing and applying biomarkers in assessing ecological risks. These studies were selected in order to showcase successes, as well as to caution against wholesale adoption of biomarkers and bioindicators as inherently valuable in risk assessment.

Perceval et al. (2004) performed a field study designed to examine the potential for metallothionein (MT) and subcellular partitioning measurements to predict higher-level effects for unionid mussels (*Pyganodon grandis*) chronically exposed to cadmium in nine Canadian lakes. The lakes were selected on the basis of trophic similarity and contrasting Cd concentrations. MT concentrations were measured in the gill cytosol from collected mussels using a ^{203}Hg saturation method. Total Cd concentration in the gill cytosol was measured by inductively coupled plasma mass spectrometry. Measured eluted fractions of metal burdens were combined into a high (245-18 kDa), an MT-like (18-1.8 kDa), and a low (<1.8 kDa) molecular weight pool. Estimates of population growth parameters, fecundity, biomass, density, and secondary production were obtained for these mussels. Food availability, the presence of mussel predators, and standard physical-chemical limnological factors were also measured in the nine lakes. Perceval et al. (2004) determined that Cd concentrations in the gill cytosolic high molecular weight pool were most frequently and strongly correlated with the measured population parameters. However, the ability of this biomarker to characterize the overall status of the mussel population appeared minimal, owing largely to confounding effects of the other ecological and limnological factors, particularly the number of accumulated degree-days. The authors also suggested that the reduced power of the Cd biomarkers in predicting the ecological status of the mussel population might have been influenced by a general decrease in Cd concentrations in the nine lakes during the two-study study.

Nevertheless, the difficulties summarized by Perceval et al. (2004) do not reflect the overall degree of success expected in the development and application of biomarkers. For example, Ricketts et al. (2003) posited that new technologies stemming from genomics research hold promise for the development of effective biomarkers, although these investigators recognized the challenge that remains to link novel molecular indices to ecologically relevant whole-organism life-cycle endpoints. To examine this promise, Ricketts et al. designed a study that examined the validity of annetocin gene expression as a biomarker for the effects of metal exposure on earthworm (*Eisenia fetida* Lumbricidae) reproduction. Annetocin elicits egg-laying behavior in *E. fetida*. The authors developed annetocin gene-specific primer pairs that showed maximum expression in earthworm tissues and segments involved in sexual reproduction. Importantly, the expression of annetocin was reduced 20-fold in earthworms exposed to mining soils contaminated with lead and zinc compared to uncontaminated reference soils. Cocoon production by the earthworms exposed to these metals was similarly decreased. Direct evidence has been provided that functionally links annetocin and reproduction in *E. fetida* (Oumi et al. 1996): A peptide that encodes the nine amino acids of annetocin induces basic reproductive events in sexually mature earthworms. Thus, Ricketts et al. (2003) appears as one of the first studies that demonstrates the expression of an invertebrate neurohormone in relation to population-level

impacts via reproductive impairment. Further elaboration of these findings aimed at constructing a stress-response function would provide the kind of risk characterization capability necessary for assessing risks for this sentinel organism in terrestrial systems.

Schlenk et al. (1996a,b) were successful in establishing a negative correlation between hepatic cytochrome P450 1A (CYP1A) and a fish health index for female largemouth bass collected from Bayou Bartholomew, Arkansas. The bayou flows through six Arkansas counties that are characterized by intensive cotton and soybean agriculture. The fish health index is based on numerical measures of 14 attributes measured for various tissues and organs. The fish health index was significantly related to species richness in this bayou. Similar correlations between biomarkers and measures of population and community-level impacts were suggested for largemouth bass, bluegill, carp, and crappie. However, although these correlations were often high, the analyses were not all statistically significant. A fair interpretation of these studies is that the biomarkers were significantly correlated with measures of whole-animal health (i.e., the fish health index), and that the whole-animal measures were at least associated with selected population and community metrics.

Another encouraging example of biomarkers potentially advancing capabilities in ERA derives from the work of Theodorakis and Shugart (1998a,b; 1997). These investigators, through a series of studies, have been able to estimate the impacts on survivorship and fecundity of mosquito fish (*Gambusia affinis*) to ionizing radiation exposures via DNA strand breakage. Methods of randomly amplified polymorphic DNA (RAPD) and allozyme analysis showed that mosquito fish exposed to radiation in the laboratory had elevated numbers of RAPD bands and increased frequency of certain bands that were related to DNA strand breakage. These authors were able to relate an indicator of strand breakage (median molecular length – MML) to changes in fecundity and survivorship for these fish. Importantly, the relationships between MML and these demographic parameters seemed to hold for populations of mosquito fish collected from geographically distant locations of radioactive contamination. Based on these studies, it appears feasible to develop a simple demographic population model that could be used to extrapolate the effects of ionizing radiation, through measures of DNA strand breakage, to changes in population size for exposed mosquito fish. This fish species offers the advantage of being a commonly used toxicity assay species and considerable information concerning reference population dynamics might be exploited to develop such a model.

Figure 2 illustrates a hypothetical biomarker-based ERA methodology derived from the preceding work with mosquito fish. Increasing exposure (Gy) to ionizing radiation manifests as increased DNA strand breakage, measured as decreases in MML of the DNA molecules (Figure 2a). Reductions in MML were correlated to reduced adult fecundity (Figure 2b) and reduced survivorship of newborn mosqui to fish (Figure 2c). These exposure-response functions enter directly into a simple two-stage demographic model that describes the population dynamics of this comparatively short-lived species (Figure 2d). The model translates reductions in adult fecundity (F_2) and reduced survivorship of newborns (P_1) to projections of future population size. The model can be implemented in a stochastic manner, where uncertainties associated with the exposure-response functions result in distributions of the fecundity and survivorship parameters. Repeated simulations (e.g., Monte Carlo methods) can produce estimates of the probability (i.e., risk) of different magnitudes of population reduction. These probabilistic risk estimates can be used to characterize and compare risks to mosquito fish populations at different sites of exposure (Figure 2e). Figure 2 demonstrates the feasibility of integrating the results of separate, related studies using biomarkers (MML) and bioindicators (fecundity, survivorship) into

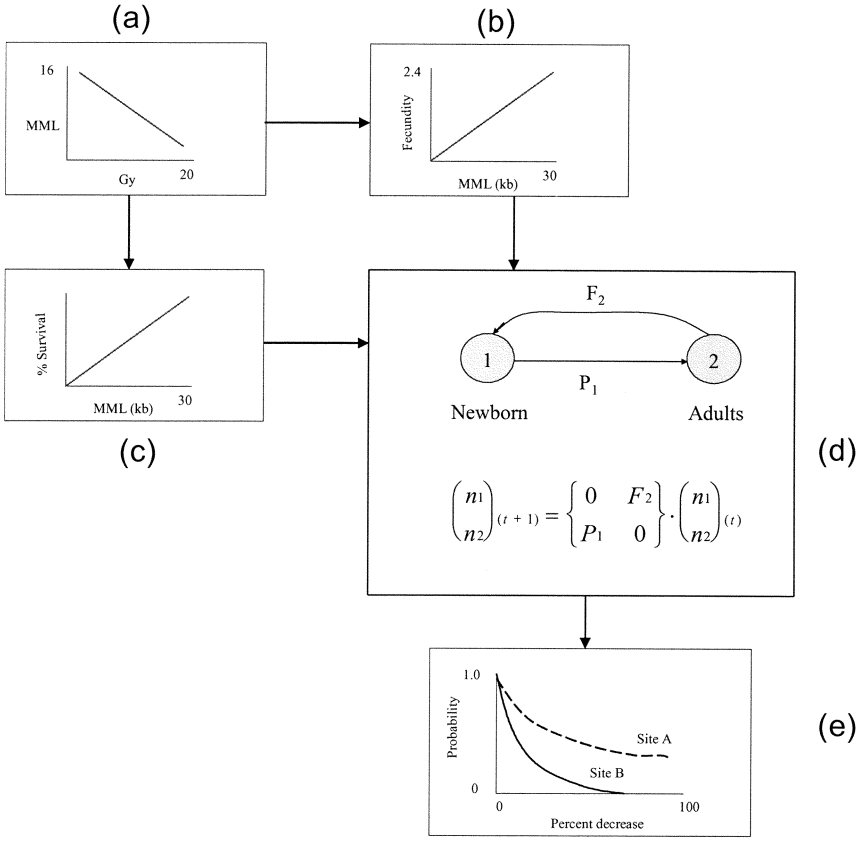

Figure 2. Schematic illustration of risk assessment model based on DNA strand breakage associated with ionizing radiation. (a) Median molecular length (MML) reflects the intensity of DNA strand breaks as a function of exposure (Gy). (b) Decreased MML is correlated with reduced adult fecundity (F_2). (c) Reductions in MML are also correlated with decreased survival of newborn (P_1). (d) The two-stage population model can be used to project population changes and (e) probabilities (i.e., risk) of different percentage decreases in sites (e.g., A, B) subject to different intensities of exposure to ionizing radiation.

an operational framework for estimating ecological risks posed, in this case, by ionizing radiation. It might prove possible to increase the set of such risk assessment tools through a careful and comprehensive review and evaluation of the biomarkers and bioindicators literature – especially if performed within the context of currently available ecological modeling capabilities (e.g., individual-based models, population models).

In another study involving mosquito fish (*G. holbrooki*), Tatara et al. (1999) attempted to correlate changes in allele frequencies for glucosephosphate isomerase (*Gpi*) allozymes with reproductive impacts measured over several generations of fish exposure to mercury in laboratory mesocosm experiments. This study produced statistically significant shifts in allele frequencies between control and treatment mesocosms. However, there were no significant relationships between these shifts in gene frequency and fish length, weight, or sex ratios. Although the authors remained optimistic about the potential for the *Gpi* marker to indicate population-level effects of long-term, low-level exposures

to contaminants, they further cautioned that the successful application of this technology would benefit from detailed understanding of the genetic and ecological history of the exposed populations. Allozyme genotoxic effects ascribed to chemical stressors can be confounded by several abiotic and biotic factors, including temperature, dissolved oxygen, predation, competition, founder effects, migration, and preexisting genetic clines (Newman and Jagoe 1998). In support of this technology, Tatara et al. (1999) maintained that many of these confounding issues can be alleviated through the use of controlled mesocosm studies.

Discussion

The protection and management of ecological resources generally focus on responses measured at higher levels of organization (e.g., USEPA 1998). Thus, the success of biomarkers in supporting ecological risk assessment depends importantly on the identification of valid biomarkers and the establishment of process-level linkages between biomarkers and higher-level responses, for example, bioindicators (Adams 2003).

A basic premise underlying the use of biomarkers and bioindicators in ecological risk assessment is that responses to chemical stress manifest initially as disruptions of normal molecular, biochemical, or physiological structure and function. If the accumulation of a toxic chemical is sufficient in magnitude and/or duration to overwhelm the normal homeostatic capacity or repair mechanisms of these biological systems, deleterious effects might be observed for individual organisms. If a sufficient number of organisms are impacted, the response to stress might be subsequently measured as changes in population size or alterations in community structure (Adams 2003). In the vernacular of hierarchy theory, the expression of stress has its explanation in levels of biological organization below its observation, and significance in levels above (O'Neill et al. 1986; Allen and Starr 1982). Therefore, studies that characterize ecological responses to chemical stressors across several levels of biological and ecological organization are particularly valuable in that such studies might identify mechanistic linkages between lower-level responses (biomarkers) and relevant individual-, population-, or community-level assessment endpoints.

The selected example studies described in relation to risk characterization were not intended as a comprehensive survey of the potential contributions of biomarkers and bioindicators to ecological risk assessment. Rather, these investigations were presented as an indication of the mixed results in attempting to incorporate advances in molecular and cellular processes – as examples of successes and failures – and to point out promising avenues for research in promoting the use of biomarkers in ERA. A comprehensive examination and evaluation of the biomarker and bioindicators technical literature could be used to construct a web-enabled "knowledge management system" (KMS) (Richards et al. 2001). A carefully designed KMS could assist risk assessors in (1) keeping currently informed on the availability of biomarkers and bioindicators with proven applicability to ERA, (2) selecting biomarkers and indicators appropriate for specific stressors and endpoints, and (3) properly implementing selected markers and indicators in specific assessments. The decision support system described in relation to problem formulation could serve as a subset of the KMS. Importantly, the KMS also could serve as nexus for developing a collective history of the successes and failures in using biomarkers and bioindicators in ecological risk assessments.

The evaluation of the potential of biomarkers to support ERA is not original to this author. For example, a conference was convened at the Centre for Environmental Toxicology

in Christchurch, New Zealand, 14–16 July, 1999 to make recommendations concerning the inclusion of biomarkers in assessing ecological risks (Adams et al. 2001). The conference delegates outlined the need to develop an experimental framework for biomarker studies that conforms to the basic guidelines suggested for assessing ecological risk (e.g., USEPA 1998).

Recommendations concerning the effective application of biomarkers in assessing ecological risks were produced by the conference participants. The delegates recognized that biomarkers could be used to support the risk assessment process in several ways Table 4. One set of recommendations addressed the continued development and evaluation of biomarkers, including the need to (1) validate biomarkers through combinations of laboratory and field studies, (2) better quantify reference values of selected biomarkers for given ecological systems, and (3) better understand the physical, chemical, and biological factors that can influence the measurement and interpretation of biomarkers. These recommendations are consistent with the desirable attributes of biomarkers and bioindicators for assessing ecological risk assessment as described and discussed in this essay – namely those attributes necessary for the development of exposure-response functions. The Christchurch recommendations also addressed the use of biomarkers to signal the exceedance of critical physiological threshold values. In the context of this current discussion, the suggested threshold values considerations are consistent with exposure-response functions that require some non-zero measure of exposure in order to manifest a response. Beyond risk assessment, the recommendations included the suggestion that biomarkers and bioindicators be pursued as measures of environmental health (Table 4). Used in this way, biomarkers and bioindicators could contribute to risk management.

A second set of recommendations focused on the use of biomarkers and bioindicators in risk management and assessment:

1. Biomarkers and bioindicators should be incorporated into risk assessment frameworks using a weight-of-evidence approach based on sensitive short-term responses and longer-term ecologically relevant endpoints
2. Biomarkers need to be related to responses of concern and then used to evaluate the safety of pesticides and other chemicals

Table 4
Uses of biomarkers in support of ecological risk assessment identified by Christchurch Conference (adapted from Adams et al. 2001)

1. Characterize mechanisms of toxicity involved in biological responses at higher levels of organization
2. Help establish causal relationships between stressors and response
3. Indicate presence of specific groups of contaminants
4. Establish absence of significant effects at population, community, or ecosystem level
5. Predict higher-level responses
6. Signal the exceedance of critical physiological thresholds or tolerance limits
7. Provide biological responses for use in weight-of-evidence approach to ecological risk assessment
8. Monitor changes in environmental health in relation to mitigation or risk management

3. Suites of biomarkers and bioindicators that address exposure and effects should be used to characterize risks posed by multiple stressors
4. Field studies should be designed to rigorously link cause (i.e., stressors) and effects measured for endpoints chosen *a priori* to represent different levels of organization
5. Novel measures that identify thresholds for environmental tolerances should be developed and incorporated into regulatory and experimental toxicology
6. Biomarkers and bioindicators should be used in assessing risks posed by agrochemicals in the context of sustainable agriculture

Regrettably, the Christchurch conference delegates concluded that despite decades of intensive research, the contribution of biomarkers to the process of risk assessment remained disappointing (Adams et al. 2001).

To the extent that the preceding recommendations can be implemented, biomarkers and bioindicators will likely increase in their usefulness for assessing ecological risk. In addition to fulfilling the preceding recommendations, risk assessors must become increasingly knowledgeable concerning the selection and application of biomarkers and bioindicators. Assessors with formal training and professional experience in more traditional ecotoxicology (e.g., acute and chronic toxicity benchmarks, effects on populations, community structure) require additional training to become more familiar with the concepts, methods, and interpretation of indicators of exposure and effects measured at suborganism levels of organization: *classical ecotoxicologists need to become better biochemists*. The KMS suggested previously could facilitate this extension of environmental toxicology to further embrace biochemistry that is relevant to risk assessment.

In turn, classically trained biochemists and physiologists, who are the principal developers of biomarkers, must become more knowledgeable concerning the potential higher-level (i.e., supraorganism) implications of biomarkers advertised as tools for risk assessment: *biochemists need to become better ecologists*. As risk assessors become increasingly comfortable and facile in considering multiple levels of biological and ecological organization while developing conceptual models, biomarkers and bioindicators will undoubtedly become more commonly and effectively incorporated into ecological risk assessments.

References

Adams SM. 2003. Establishing causality between environmental stressors and effects on aquatic ecosystems. Hum Ecol Risk Assess 9: 17–35.

Adams SM, Greeley MS. 2000. Ecotoxicological indicators of water quality: Using multi-response indicators to assess the health of aquatic ecosystems. Water Air Soil Poll 123: 103–15.

Adams SM, Giesy JP, Tremblay LA, Eason CT. 2001. The use of biomarkers in ecological risk assessment: Recommendations from the Christchurch conference on Biomarkers in Ecotoxicology. Biomarkers 6: 1–6.

Allen TFH, Starr TB. 1982. Hierarchy—perspectives for ecological complexity. Chicago: University of Chicago Press.

Bartell SM. 1996. Ecological/environmental risk assessment: Principles and practices. In: Kolluru RV, Bartell SM, Pitblado RM, Stricoff RS, editors. Risk assessment and management handbook. New York, NY: McGraw-Hill. p 10.3–10.59.

Bartell SM, Gardner RH, O'Neill RV. 1992. Ecological risk estimation. Chelsea, MI: Lewis Publishers.

Bradley BP, Shrader EA, Kimmel DG, Meiller JC. 2002. Protein expression signatures: An application of proteomics. Mar Environ Res 54: 373–7.

Burger J, Gochfeld M. 2001. On developing bioindicators for human and ecological health. Environ Monit Assess 66: 23–46.

Lorenz C. 2003. Bioindicators for ecosystem management, with special reference to freshwater systems. In: Markert BA, Breure AM, Zechmeister HG, editors. Bioindicators and biomonitors. Amsterdam: Elsevier. p 123–52.

McCarty LS, Munkittrick KR.. 1996. Environmental biomarkers in aquatic toxicology: Fiction, fantasy, or functional? Hum Ecol Risk Assess 2: 268–74.

Moore MN, Depledge MH, Readman JW, Leonard DRP. 2004. An integrated biomarker–based strategy for ecotoxicological evaluation of risk in environmental management. Mutat Res 552: 247–68.

Newman MC, Jagoe RH. 1998. Allozymes reflect the population-level effect of mercury: Simulations of the mosquitofish (*Gambusia holbrooki* Girard) GPI-2 response. Ecotoxicology 7: 141–50.

Ogunseitan OA. 2000. Microbial proteins as biomarkers of ecosystem health. In: Scow KM, Fogg GE, Hinton DE, Johnson ML, editors. Integrated assessment of ecosystem health. Boca Raton, FL: Lewis Publishers. p 207–22.

O'Neill RV, DeAngelis DL, Waide JB, Allen TFH. 1986. A hierarchical concept of ecosystems. Princeton, NJ: Princeton University Press.

Oumi T, Ukena K, Matsushima O, Ikdea T, Fujita T, Minakata H, Nomoto K. 1996. Annetocin, an annelid oxytocin-related peptide, inducing egg-laying behavior in the earthworm, *Eisenia foedita.* J Exp Zool 276: 151–156.

Perceval O, Couillard Y, Pinel-Alloul B, Giguere A, Campbell PGC. 2004. Metal-induced stress in bivalves living along a gradient of Cd contamination: Relating sub-cellular metal distributions to population-level responses. Aquat Toxicol 69: 327–45.

Potyrailo RA, Conrad RC, Ellington AD, Hieftje GM. 1998. Adapting selected nucleic acid ligands (aptamers) to biosensors. Anal Chem 70: 3419–3425.

Richards DJ, Allenby BR, Compton WD. 2001. Information systems and the environment. Washington, DC: National Academy Press. 228 p.

Ricketts HJ, Morgan AJ, Spurgeon DJ, Kille P. 2003. Measurement of annetocin gene expression: A new reproductive biomarker in earthworm toxicology. Ecotox Environ Safe 57: 4–10.

Schlenk D, Perkins EJ, Layher WG, Zhang YS. 1996a. Correlating metrics of fish health with cellular indicators of stress in an Arkansas bayou. Mar Environ Res 42: 247–51.

Schlenk D, Perkins EJ, Hamilton G, Zhang YS, Layher W. 1996b. Correlation of hepatic biomarkers with whole animal and population-community metrics. Can J Fish Aquat Sci 53: 2299–309.

Schulte PA, Waters M. 1999. Using molecular epidemiology in assessing exposure for risk assessment. Ann NY Acad Sci 895: 101–11.

Shepard JL, Bradley BP. 2000. Protein expression signatures and lysosomal stability in *Mytilus edulis* exposed to graded copper concentrations. Mar Environ Res 50: 457–63.

Shepard JL, Olsson B, Tedengren M, Bradley BP. 2000. Protein expression signatures identified in *Mytilus edulis* exposed to PCBs, copper, and salinity stress. Mar Environ Res 50: 337–40.

Suter GW Jr. 1993. Ecological risk assessment. Boca Raton, FL: Lewis Publishers. 538 p.

Tatara CP, Mulvey M, Newman MC. 1999. Genetic and demographic responses of mosquitofish *(Gambusia holbrooki)* populations exposed to mercury for multiple generations. Environ Toxicol Chem 18: 2840–5.

Theodorakis CW, Shugart LR. 1997. Genetic ecotoxicology II: Population genetic structure in radionuclide-contaminated mosquitofish *(Gambusia affinis)*. Ecotoxicology 7: 227–35.

Theodorakis CW, Shugart LR. 1998a. Genetic ecotoxicology III: The relationship between DNA strand breaks and genotype in mosquito fish exposed to radiation. Ecotoxicology 7: 227–35.

Theodorakis CW, Shugart LR. 1998b. Natural selection in contaminated environments: A case study using RAPD genotypes. In: Forbes VE, editor. Genetics and ecotoxicology. Philadelphia, PA: Taylor and Francis. p 123–49.

United States Environmental Protection Agency (USEPA). 1998. Guidelines for ecological risk assessment. PA/630/R-95/002F, Risk Assessment Forum. Washington, DC: USEPA.

World Health Organization (WHO). 2001. Environmental Health Criteria 222. Biomarkers in risk assessment: validity and validation. Geneva, Switzerland: World Health Organization.