Environmental Toxicology and Risk Assessment

Biomarkers and Risk Assessment

Fifth Volume

David A. Bengtson and
Diane S. Henshel, editors

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Foreword

This publication, *Environmental Toxicology and Risk Assessment: BioMarkers and Risk Assessment—Fifth Volume*, contains papers present at the symposium of the same name, held on 3-5 April 1995 in Denver, Colorado. The symposium was sponsored by ASTM Committees E-47 on Biological Effects. David A. Bengtson of the University of Rhode Island in Kingston, RI and Diane S. Henshel of Indiana University in Bloomington, IN presided as symposium chairpersons and are editors of the resulting publication.

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Overview

This volume includes papers presented at the ASTM Fifth Symposium on Environmental Toxicology and Risk Assessment, held in April 1995, in Denver, Colorado and sponsored by ASTM Committee E-47 on Biological Effects and Environmental Fate. The theme of the Symposium was Biomarkers and Risk Assessment. From the total of more than 100 oral and poster presentations at the Symposium, this volume represents a select few for which manuscripts were submitted and subjected to a rigorous peer-review process. As with the previous four ASTM Symposia on Environmental Toxicology and Risk Assessment, presentations at the meeting included a mixture of theme-session and non-theme contributions. The contributions to this volume are similarly divided between papers on the biomarker theme and those on general environmental toxicology and risk assessment.

The goals of the plenary session and the several biomarker-focused sessions throughout the Symposium were both to explore the usefulness of established biomarkers and to identify new biomarkers that are under development. A critical question is "How might biomarkers be useful in the future in the environmental and risk assessment processes?"

What is a biomarker? As defined by ASTM, a biomarker is 'a biological measure (within organisms) of exposure to, effects of, or susceptibility to environmental stress using molecular, genetic, biochemical, histological or physiological techniques." Thus, biomarkers are generally sublethal changes. Ideally, they should be consistently quantifiable (that is, the measured results should be readily replicable). The quantifiability of different biomarker techniques varies. Histopathological markers tend to be more qualitative, whereas biochemical and physiological markers tend to be very quantitative. Biomarkers are being developed at several levels of biological organization. Those at higher levels (anatomical or physiological) are presumed to integrate changes occurring at lower levels of organization (molecular or cellular). One of the most important challenges of biomarkers research is to understand the mechanisms of change at a given level and to then understand whether and how those changes are integrated at the next higher level.

Why use biomarkers in environmental and risk assessments? Classical endpoints used for risk assessment, for example, mortality or tumor induction, are either too severe or take too long to develop. Using death as the endpoint to establish safe levels of exposure leaves very little margin for the variation in sensitivity between individuals in a species or between species. We now estimate safe levels based on some safe or acceptable exposure level, a no-effect level or an effective dose for 10% of the population (ED10), integrating uncertainty and judgment factors into the equation. If our margin of safety is wrong for some untested population, we have allowed the possibility of a lethal effect in some percentage of a particularly sensitive population. If, on the other hand, safe levels are established based on more subtle, sublethal endpoints, such as biomarkers, behavior, or other biological indicators, then even super-sensitive populations will be better protected from such severe effects as increased mortality. In addition, toxicity testing for exposure and effects should be as cost-effective and time-efficient as possible, because many chemicals and sites must be tested with limited funding. Standard testing for cancer (tumorigenesis) is very costly because it requires a large number of animals to be maintained under test conditions for a large portion of their lifespan. Many short-term, biomarker-based mutagenicity tests have been developed and more are in development. Each such test has its limitations and

appropriate uses, but a battery of such tests can be used to screen chemicals that require further testing as potential mutagens or carcinogens to target animal species. Another example of the potential uses of biomarkers is in the relatively new field of endocrine disruption. Until recently, chemicals that disrupt the endocrine system were only identified during testing for reproductive effects, which is also a costly endeavor in terms of time and money. More rapid and less costly screening techniques have recently been developed to assess the potential for chemicals to affect the endocrine system. It is becoming clear that some chemicals disrupt parts of the endocrine system beyond those involved in reproduction. Biomarker-based assays are facilitating the search for other endocrine-disrupting effects of environmental compounds (natural and anthropogenic). In the long term, there will be a need for molecular-level biomarkers that allow determination of "effect" and "no effect" levels for chemicals that will be protective of even sensitive populations.

The three invited speakers in the plenary session, Drs. John Stegeman, John McLachlan and Steven Bartell, discussed biomarkers and their use in the environmental and risk assessment process. Dr. Stegeman discussed many types of biomarkers, especially some of the more well-established biochemical and molecular markers now in use. He pointed out that each marker (measurement) has its own unique utility and pitfalls. Each measurement has a different time course, a different sensitivity and something separate to contribute to our understanding of a process, such that maximum information about an exposure is gained when several measurements are made in concert. Several factors must be considered when developing biomarker-based assays, such as the relative species and chemical specificities of the endpoints being measured; these specificites can often be determined empirically. Understanding the mechanisms controlling the interaction of the chemical with the measured endpoint helps to identify the potential functional significance of changes in that endpoint. Further understanding mechanisms under different physiological conditions (including such natural influences as daily or seasonal homeostatic fluctuations) improves our ability to interpret a given biomarker endpoint. Dr. Stegeman further pointed out that one needs to understand the causes of both increases and decreases in the signal that one measures in order to adequately interpret the measurements. Once the measurements are made, their biological implications for the animal must be understood. The effects measured must be interpreted within the context of the animal or species to respond. Determination of linkages between the measured biomarker effects and biologically significant effects at the organismal and population levels represent a research challenge for the future.

The second plenary speaker, Dr. McLachlan, addressed the need for standardization in biomarker-based assays. Given the important role that standardization generally plays in research, and the fact that we have no real standards as yet in the biomarker arena, it is time to focus on the devlopment of standards. Biomarker assays are based on perturbations in the normal homeostatic mechanisms of the body and are useful specifically because there are interactions between chemicals and cells and chemicals in cells, between cells in tissues, between tissues in organs, between organs in organisms, between organisms in populations, and between populations in communities. Effects or perturbations detected at one level of organization can have and do have effects at other levels of organization. When we understand these interactions, we can better interpret the relevance of perturbations in biomarker assays to the system as a whole. Dr. McLachlan pointed out that, during the last 20 years, there has been considerable research on, and production of standards for, the interaction of the environmental agents with genetic material leading to disease and dysfunction. We know a lot about, and have tests to measure, interactions of chemicals with our genome. We have many assays to look at mutation frequnecy and to try to correlate it with a variety of dysfunctions, especially cancer. Dr. McLachlan identified a currently emerging area of research as the study of environmental agents working through signaling molecules,

not only membrane-related proteins but the whole array of gene regulation, gene expression and signals that enter the cell and result in a variety of adverse effects. He predicted that this would present a greater challenge both in terms of research and standards development than did the previous genetic research because the genetic research had "archival" material in the structure of DNA. The signaling research will be hampered by the differences in time scales over which the signaling events may occur and by the transient nature of the signals, such that they may not be detectable at the time the dysfunction is expressed. The new challenge presented by this research over the next 20 years will require as much ingenuity as has been applied to understanding the interactions of environmental agents with the genome over the last 20 years. The issue of environmental estrogens is one example of signaling mechanisms and their possible use in biomarker assays. Environmental estrogens are estrogen mimics and can be considered a metaphor for molecules in the environment that mimic our internal signaling molecules. These environmental mimics work at the interface between the internal and external environment. Environmental estrogens will be increasingly important as metaphors for understanding signaling changes in a variety of systems and we need to know more about them and the biological systems they affect.

The third plenary speaker, Dr. Bartell, discussed how the development of biomarkers, which have allowed us to characterize exposure and effects for some metals, organics and pesticides, could help us improve the ecological risk assessment process. Ecological risk assessment recognizes and attempts to identify, quantify and propagate all of the uncertainties inherent in the analysis and to express the results of the assessment as a probabilistic term based on those uncertainities. Dr. Bartell pointed out that biomarkers could help us with both exposure assessment and response with regard to the dose-response components of the ecological risk estimation. Biomarkers can indicate exposure to, or effects of, an environmental agent, but they cannot by themselves indicate what changes have been imposed upon the ecosystem as a whole. However, if we could develop relationships between intensities of different biomarkers and survivorship or reproduction probabilities, we could extrapolate from the biochemical level up to the population level. By understanding the linkages between cellular, organismal, and population levels, we could use biomarker results to predict probabilities at the higher levels of organization. The process of researching and understanding these linkages would very likely enable us to better understand the complexity of ecosystems generally. Thus, biomarkers might provide a catalyst for a more general understanding of the relationships between different levels of biological organization through the analysis of the propagation of perturbations through systems. Dr. Bartell concluded his remarks with the opinion that there is no inherent reason why we could not ultimately make intelligent decisions on regulation of contaminants directly from biomarkers, because we don't necessarily have to focus on population, community, or ecosystem level impacts. If the scientific basis is available, we could decide to use biomarkers as valid endpoints for decision making. Some biomarkers may be most useful in ecological risk assessment in the context of evaluation of sites with multiple contaminants. If biomarkers could be used to conduct an initial screening to help narrow the scope of the problem, so that attention could be focused on the contaminants posing the greatest risk, that could be a very beneficial use of biomarkers in risk assessment, in Dr. Bartell's opinion.

The biomarkers section of this volume includes papers on a variety of biomarker responses, including molecular, cellular, genetic, developmental and neurotoxicological, measured in a variety of organisms from bacteria to humans. Some of these biomarkers are well established and have been tested in a number of species. Others are still being developed. The first two papers (Palmer & Selcer and Denslow et al.) address the use of vitellogenin as a biomarker for environmental estrogens and therefore contribute to the growing body of knowledge on the effects of endocrine disrupting chemicals and potential biological indica-

tors that may be used to assess exposure to these chemicals. Vitellogenin appears to be one such biomarker that will be useful across a variety of submammalian species. The next paper (Hewitt et al.) discusses findings that might also be related to reproductive problems in fish. Anderson et al. and Melancon describe the usefulness of assessing the induction of specific cytochromes P450 as biomarkers of exposure to specific classes of chemicals, including many PAH's, as well as laterally substituted chlorinated dioxins and PCB's. The reporter gene system described by Anderson et al. is currently under consideration for inclusion as an ASTM standard. A series of three papers (Lucas & Straume, Law et al., and Donnelly et al.) on genotoxicity follows, describing biomarkers that are being developed as indicators of exposure and effects and providing evidence of genetic damage due to both chemical agents and radiation. This section on cellular- and molecular-level biomarkers closes with a set of papers on smoke exposure in humans (Rees et al.), aromatic hydrocarbon toxicity in plants (Gensemer et al.), identification of proteins (Bradley et al.) and toxic effects in cladocerans (Fort et al.). The Gensemer et al. paper is noteworthy in its correlation of the biomarker endpoint with a population-level effect. Fort et al. demonstrate that subtle changes in membrane potential are clearly biomarkers of functional effect at the cellular level. The next set of three papers (Fort and Stover, Dickerson et al. and Henshel) explore embryological development as an integrator of cellular-level effects of toxicants on organisms. Embryonic development is a very sensitive life stage and there are relatively few well-established biomarkers to assess adverse developmental effects. The FETAX assay, which is extended in the paper by Fort and Stover, is one of the better-tested biomarkers of effects on embryonic development in field situations. The early embryo assay, discussed in the papers by Dickerson et al. and Henshel, is now being developed for future use in field assessments. Finally, the last two papers in the Biomarkers section (Henshel et al. and Eells et al.) examine potential biomarkers of neurotoxicity in birds and mammals. Henshel et al. provide information on a biomarker for developmental neurotoxic effects, for which there is a paucity of biomarker assays at present. The papers by Eells et al. and Henshel discuss the critical question of the use of appropriate animal models in the devlopment of biomarkers; use of an appropriate model may determine whether the biologically relevant effect (retinal degeneration induced by methanol exposure in the case of Eells et al.) will be observed or not. The question is important when one tries to mimic effects seen in a particular species, for example, humans, because biochemical differences among species have been well documented. The Eells et al. paper especially emphasizes the importance of understanding the mechanism underlying the change measured as an effect.

The papers presented in this section thus represent an up-to-date, broad survey of the several classes of biomarkers that are currently being studied and the several classes of vertebrates and invertebrates in which these methods can be evaluated.

The second section of this volume contains papers from the Symposium that do not include biomarkers among the techniques used. The first two papers are in the field of aquatic toxicology. Lussier et al. provide information that can be used to improve an existing ASTM standard on life-cycle tests with saltwater mysids. Lytle and Lytle then present results from toxicity tests with a salt marsh macrophyte, which represents a group of organisms relatively little tested in comparison to their importance in the environment. The next two papers (Pinza et al. and McCauley et al.) deal with problems (ammonia in sediments and porewater extraction, respectively) that have vexed sediment toxicologists for years. The three papers on beahvior (Lipton et al., Nepomnyashchikh et al. and Misra et al.), each of which is interesting in its own right, provide a useful reminder of the connection of biomarker parameters to higher levels of biological factors, such that behavioral changes can be used as an indicator of functional effect; both behavior and biomarkers are most useful when they yield information about sublethal effects of toxicants. Indeed, behavior-based

assays are being developed for use in toxicity assessments for application to risk assessment. Harrass and Klemm and Hall provide excellent reviews and valuable advice on quality assurance and toxicity identification, respectively, in the context of laboratory toxicity testing. Peterson and Knowlton then describe a computer-based risk assessment system and Mahoney et al. discuss the difficulties for risk assessors when dealing with different forms of chromium in the environment. Finally, papers by Hsu and Yeung and Li and Yeung provide a mathematical model and a new method of data interpretation, respectively, for engineering problems associated with volatile organic compounds (VOC), although these papers may have more general relevance to those outside the VOC field as well. The papers in this second section thus provide a potpourri of interesting and valuable information ranging over the broad spectrum of the field of environmental toxicology and risk assessment.

We wish to thank both the authors and reviewers of the papers for their considerable efforts, the editorial staff of ASTM (especially Shannon Wainwright) for their constant help, and Kenneth St. John of the ASTM Committee on Publications for continually pushing us to meet deadlines.

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