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The use of biomarkers in ecological risk assessment: recommendations from the Christchurch conference on Biomarkers in Ecotoxicology

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Introduction

A conference to assess and evaluate biomarkers in environmental protection was organized by the Centre for Environmental Toxicology (CENTOX) in Christchurch, New Zealand, 14–16 July, 1999. The aims of the meeting were to assess the current status of the application of biomarkers, to identify emerging concepts for their practical use in environmental bioassessment, and to raise recommendations for ensuring their continued inclusion into ecological risk assessment (ERA). Biomarkers are extremely topical in ecotoxicological research as they provide functional measures of receptor species, exposure to environmental stressors that can be better related to the adverse effects of human activities. This effects-based information can be used in support of environmental management and regulation.

Definitions

In general, biomarkers can be characterized as functional measures of exposure to stressors, which are usually expressed at the suborganism level of biological organization (Benson and Di Giulio 1992, Huggett *et al.* 1992, Depledge and Fossi 1994). Bioindicators, on the other hand, are defined less precisely and can be viewed as either structural entities such as sentinel species (Van Gestel and Van Brummelen 1996), or they can be considered functionally as biological-effects end-points at higher levels of organization (Adams 1990, Engle and Vaughan 1996). McCarty and Munkittrick (1996), however, have related the concept of biomarkers and bioindicators in one definition, which considers bioindicators as, 'anthropogenically-induced variation in biochemical, physiological, or ecological components or processes, structures, or functions (i.e. a biomarker) that has been either statistically correlated or causally-linked, in at least a semiquantitative manner, to biological effects at one or more of the organism, population, community, or ecosystem levels of

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biological organization'. In the field of ecotoxicology, biomarkers and bioindicators have been defined in many different ways and used in an extensive array of ecotoxicological investigations. For the sake of clarity, the conference delegates agreed that it is important to clearly define the operational definitions of these terms within the context of each specific use and study objective.

Applications

The potential utility of biomarkers in environmental ecotoxicology was initially promoted in the early 1990s (Adams 1990, McCarthy and Shugart 1990, Niimi 1990, Peakall 1994a,b). Although biomarkers have been historically most successful in identifying contaminated areas and potential chemical stressors, in some cases they have been inappropriately used or the results misinterpreted. The accuracy, precision, and validation of biomarkers continues to improve as the understanding of the mechanisms of toxicity increases and causal relationships are identified and established between exposure responses to environmental stressors and whole animal/population effects. Recent developments in molecular genomics and proteomics along with the linking of biological systems with microelectronics should provide increasing opportunities to develop more powerful biomonitoring techniques to manage and protect the integrity of ecosystems. There is clearly a need for the continued development, validation, and improvement in the application of biomarkers, particularly as many environmental agencies are now recognizing the importance of utilizing these multi-response end-points within biomonitoring and assessment programmes and within the ERA framework.

Biomarkers can provide a functional measure of organism response exposed to both single and complex mixtures of chemical stressors that are bioavailable in the environment. The types of biomarkers available range from (1) general (e.g. stress proteins) to specific (e.g. acetylcholinesterase), (2) relatively low sensitivity (e.g. histopathology) to high sensitivity (e.g. cortisol), and (3) molecular to the individual level of biological organization.

In general, biomarkers can be classified into three groups, similar to the standards described by Peakall (1994a), depending on their level of demonstrated use in laboratory and field situations and their level of validation. Validation in the context of these Peakall (1994a) standards relates to critical mechanisms of action, standardization of methodologies, and field testing under controlled and field situations. This is, however, a rather general and broad definition of validation and we prefer to consider validation as a process of establishing mechanistic or causal relationships between exposure to environmental stressors and the significant biological or ecological changes of interest. Examples of the most widely applied biomarkers that are in various stages of validation through extensive field applications include the heat shock proteins (HSP), the mixed function oxygenases (MFOs) detoxifying enzymes, the metallothionein enzymes, bile metabolites, and oxidative stress enzymes (e.g. superoxide dismutase). A second group that has been used rather extensively in field applications but has yet to be validated to the level of this first group includes measures of genotoxicity (DNA adducts, strand breaks) and end-points of population genetic diversity, immunotoxicological responses, and many of the reproductive impairment measurements such as plasma vitellogenin and plasma sex steroid hormones. A third group that holds particular promise but has not been applied to any significant degree in field studies includes

new emerging biomarkers such as electrophysiological responses, gene expression proteins, lysosomal membrane integrity, and the development of biosensors.

Overall, the conference delegates recognized that biomarkers can be useful for addressing a variety of issues depending on the purposes and needs of a particular study. Some of the main uses identified are:

1. To characterize the mechanisms of toxicity of stressor(s) involved in biological responses at higher levels of organization.
2. To help establish relationships between cause (stressor) and effect (response).
3. To indicate presence/absence of specific groups of contaminants.
4. To establish absence of significant biological or ecological effects at the population, community, or ecosystem level.
5. To predict higher-level responses (for biomarkers that have been at least semi-quantitatively related to higher levels of organization).
6. To signal whether critical physiological thresholds or tolerance limits have been exceeded.
7. To provide a range and diversity of biological responses that could be used as a weight-of-evidence approach in the ERA process.
8. To monitor environmental health and document improvements following risk management or mitigation.

The selection and application of biomarkers needs to be made in the context of the issue or objectives of concern. A number of factors, ranging from physico–chemical to biological variables, need to be considered when selecting the appropriate measures of exposure. The delegates recommended that an experimental framework of biomarker studies should be developed that applies to each situation and that this general framework should conform to the basic guidelines suggested in the Framework for Ecological Risk Assessment developed by the US-EPA (1998). A generalized field-biomonitoring framework should consist, for example, of a weight-of-evidence approach, applying lines of evidence at several different levels of biological organization. The appropriate biomarker(s) would then be selected based on the information available. Depending on the amount of information available for a particular site, the proportion of specific vs non-specific and sensitive vs non-sensitive biomarkers applied will vary. For example, if a reasonable amount of *a priori* knowledge is available for a particular ecosystem, such as the physico-chemical characteristics, ecological structure, and associated environmental stressors, a smaller suite of non-specific and non-sensitive markers can be applied. In contrast, for ecosystems with a relatively low amount of available information on these characteristics, a larger variety and suite of biomarkers may have to be included in the experimental design. Therefore, the design of an appropriate bioassessment programme may include several phases or stages progressing over time from a proportionally larger number of non-specific and less sensitive markers to a reduced set of more specific and sensitive end-points as additional biological and physico-chemical information becomes available. Particular biomarkers would also be selected based on the known toxicological properties of the contaminants or the expected responses of the organism to particular stressors. The delegates endorsed the adoption of the selection criteria suggested by McCarty and Munkittrick (1996) where a biomarker can be considered a bioindicator of high-level effects if that biomarker can be at least semi-quantitatively linked to an ecologically relevant end-point. The delegates also

suggested that the application of biomarkers in field studies would be more successful if dose–response relationships were adequately developed, first in the laboratory, between the stressor of interest, specific measures of exposure, and a bioindicator of a biologically significant effect. Toward this end, the application of the criteria for establishing causality (Soimasuo *et al*. 1995, Adams *et al*. 1996, Attrill and Depledge 1997, Gilbertson 1997) and the ecoepidemiological criteria suggested by Fox (1991) are useful guidelines.

In addition to demonstrating effects, appropriately selected and applied biomarkers can be used to establish the lack or absence of effects. While it is difficult to demonstrate a negative or absence of effects, it is not entirely impossible. To be confident in the conclusions of such an assessment, the selected biomarker(s) must be sufficiently sensitive and the statistical power of its measurements adequate to help establish possible linkages between the biomarker and the responses of concern. It is imperative that appropriate statistical tests be chosen in the initial experimental design to fully assess the relationships between individual biomarkers and responses at higher levels of biological organization.

Recommendations

Provided below is a list of recommendations and challenges for the effective application and use of biomarkers within the ERA framework. This first set of recommendations is provided to encourage and stimulate the continued development, advances, and practical application of biomarker technology in environmental health assessment:

1. Validation of biomarkers through combined laboratory and field studies. Such work is needed to understand mechanisms of effects and to link cause (stressor) to exposure responses and ultimately to ecologically relevant end-points.
2. Better characterization of the 'normal state' of a given system, which is not necessarily the average state but rather the ranges of variation, to help define the limits of a response that must be exceeded in order to cause an adverse biological effect.
3. Better identification and understanding of modifying/modulating factors (i.e. habitat and food availability, other physico-chemical factors, density-dependent interactions such as abundance, behavioural considerations, etc.) that influence the interpretation/evaluation of biomarker responses to stress at various levels of biological organization. This would also include the relative role and importance of spatial and temporal variability in the proper assessment, interpretation, and evaluation of biomarkers data.

A second set of recommendations addresses how biomarkers could be used more effectively in risk management and assessment:

1. Biomarkers/bioindicators should be incorporated into the ERA frameworks using a suite of sensitive short-term responses and also longer-term ecologically relevant end-points that provide a weight-of-evidence approach for establishing relationships between environmental stressors and ecological effects.
2. Once they are linked to responses of concern, biomarkers should play an increasing role in the safety evaluation of pesticides and other chemicals by contributing effects-based information.

3. A proper suite of biomarkers/bioindicators reflecting both exposure and effects (and modes of action) of suspected stressors such as contaminants should be used to characterize systems subjected to multiple stresses. Where appropriate, chemical analysis of representative media (tissue, water, sediment) should be used to complement biomarkers of exposure.

4. The experimental design of field studies should focus on multiple end-points representing various levels of biological organization in order to increase the probability of linking cause (e.g. the stressor) to the effect of concern. The major responses or effects of concern should be identified *a priori* such as reproductive or population-level end-points.

5. Novel measures of exposure in wildlife species as they become available should be incorporated into regulatory and experimental toxicology to help determine threshold levels for environmental tolerances.

6. Biomarkers/bioindicators should be increasingly used in contaminant risk management and to validate 'green' agroecosystem practices aimed at reaching sustainability (Nychas 1995, Wratten *et al*. 1997).

Conclusions

In general, the suite of biomarkers applied in field bioassessment studies should conform to the needs of the risk assessor and risk manager. The manner in which the results are to be applied to environmental assessment and to associated measurement end-points should be explicitly stated before such field investigations are initiated. Current consensus is that, in large-scale ERAs, future biomarker studies should focus on establishing the relationships to effects at ecologically significant end-points such as at the reproductive, population, and community levels. However, in some instances (i.e. monitoring effluent and waste toxicity at specific sites), these changes in feral or surrogate species or *in vitro* test systems or biosensors do not need to be linked to ecologically relevant end-points. In such cases, indicators of health effects or contaminant exposures may be sufficient to trigger a management response or further studies.

Overall, the conference delegates concluded that, after more than a decade of intensive research, the integration of biomarkers within the process of risk assessment has been somewhat of a major disappointment. A lack of clear objectives, difficulties inherent in the research design and application, and inadequate experimental design in several instances have limited the useful application and somewhat damaged the image and practical utility of this potential assessment technology within the ERA process. A major recommendation of the conference was that the 'development of biomarkers for their own sake' should be discouraged in favour of focusing future research efforts on the incorporation and application of biomarkers/bioindicators within the context of appropriate environmental bioassessment approaches such as ERA. To provide better predictive and assessment tools for environmental protection and encourage the proper use of biomarkers/bioindicators, increased integration of multidisciplinary expertise across research institutions becomes a priority if such a goal is to be achieved.

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