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# Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms

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Biomarkers have received a lot of attention but the biomarker concept has not yet been defined properly. As a consequence, various interpretations of the term 'biomarker' exist, some of which overlap with well-established ecotoxicological concepts. To allow incorporation of the biomarker concept within the framework of modern ecotoxicology, a clarification of terms is needed. In this paper, definitions are presented for the terms 'biomarker', 'bioindicator' and 'ecological indicator', which do not overlap and are linked to different levels of biological organization. The use of these concepts for ecological effects assessment is discussed.

*Keywords*: biomarkers; ecological risk assessment; bioindicator; ecological indicator.

## Introduction

The September 1994 issue of *Ecotoxicology* (Volume 3, Number 3) presented four review papers on the role of biomarkers in environmental assessment. These papers were commissioned by the European Science Foundation (ESF), which has put forward the question 'to what extent biomarkers can be used in the assessment of environmental "damage" and in the formulation of regulations to control such damage'. Discussing these papers in our working group on animal ecology at the Vrije Universiteit of Amsterdam, we came to the conclusion that the answer to this question is not straightforward because a proper definition of biomarkers is lacking. The biomarker idea has not yet crystallized into a clear concept, which seriously blocks the discussion on the possibilities and limitations of 'biomarkers'. Furthermore, the terms 'biomarker', 'bioindicator' and 'ecological indicator' seem to overlap.

The lack of proper definitions is a familiar problem in the development of scientific theories. A nice example has been given by Endler (1986). In his book on natural selection in the wild, he clearly demonstrates that the arguments and confusion on the concept of natural selection were the result of improper definitions.

The aim of this paper is to comment on the review papers in the September 1994 issue of *Ecotoxicology* and to give a clear definition of terms within the framework of modern ecotoxicology.

## The non-exclusive use of the term 'biomarker'

It is clear that biomarker research has led to a large number of promising results. The use of (non-destructive) biomarkers offers great opportunities for a fast and sensitive detection of chemical stresses within organisms (McCarthy and Shugart 1990, Fossi *et al.* 1992, Theodorakis *et al.* 1992, Walker 1992, Livingstone 1993, Peakall and Shugart 1993). These studies clearly illustrate that the concept of biomarkers is successful and deserves a place within the theoretical framework of modern ecotoxicology. Like all biological measurements, biomarker techniques integrate exposure in time and space and may avoid difficulties in the interpretation of chemical concentration measurements due to differences in bioavailability (McCarthy and Shugart 1990, Peakall 1994). These techniques give additional information on possible harmful effects 'that cannot be obtained from the measurement of chemical residue levels in environmental and biological media' (Depledge and Fossi 1994).

Koeman *et al.* (in Peakall and Shugart 1993) remarked that 'the term "biomarker" does not refer to a new concept, but is just a new name for a monitoring principle already in existence'. Adams (1990) used the term biological indicator while referring to the definition of biomarkers given by the NRC (1987). The confusion around the different concepts used by different authors is recognized by Hunsaker (1993), who used the general term 'indicators' within the framework of the Environmental Monitoring and Assessment Programme (EMAP) of the United States Environmental Protection Agency (US-EPA).

This confusion of concepts is present throughout the review papers in the September 1994 issue of *Ecotoxicology*. In his introduction to the four review papers, Peakall (1994) defined a biomarker as 'a biological response to a chemical or chemicals that gives a measure of exposure and sometimes, also, of toxic effect'. According to Peakall (1994), the levels of biological response that can be considered range from the molecular to the structure of communities and even to the function and structure of ecosystems. This is a very broad and not very exclusive definition. Peakall (1994), recognizing this, indicated that it is difficult to decide at which point the term 'biomarker' should be replaced by another term such as 'ecological indicator'. It is evident that Peakalls' (1994) biomarkers can say something about ecosystem effects.

In the same issue of *Ecotoxicology*, Peakall and Walker (1994) narrowed down the levels of biological response to range from molecular to species composition, leaving out ecosystem function as a possible level of biomarker response. Actually, they restricted their discussion mainly to biochemical markers. Peakall and Walker (1994) underlined that 'all biomarkers are biomarkers of exposure and indeed all biomarkers demonstrate an effect of some sort or another; if the term "effect biomarker" is used it should be biomarkers of *toxic* effect'.

Ernst and Peterson (1994) reviewing the literature on biomarkers in plants, limited their definition of a biomarker to 'biochemical, physiological and morphological changes in plants to measure their exposure to chemicals'. Thus, they only considered a biomarker to give a measure of exposure, which is reflected by changes in the organism. Yet, they believed that 'for ecotoxicology to progress, biomarkers of various types ... will be needed to evaluate chemical hazards to species, communities and ecosystems ...' (Ernst and Peterson 1994). In fact, they accept the use of biomarkers for the assessment of effects (which result from exposure).

Discussing the use of biomarkers for the prediction of effects on invertebrate populations and communities, Lagadic *et al.* (1994) clearly distinguished between biomarkers and bioindicators. They restricted the use of the term 'biomarker' to 'biochemical sublethal changes resulting from individual exposure to xenobiotics' and concluded that we are still far from predicting ecosystem effects on the basis of biomarker responses in individual organisms.

Depledge and Fossi (1994) however, were more optimistic about the possibilities of predicting ecosystem responses on the basis of biomarker measurements. They also adopted the broad definition of biomarkers given by Peakall (1994), but further specified that biomarkers say something about the (departure from) health of individual organisms and may give some indication of their 'fitness'. From this it becomes clear that they restricted the term biomarker to measurements at or below the level of individual organisms. In an attempt to answer the question raised by the ESF, Depledge and Fossi (1994) recommended the development of a procedure for the extrapolation from 'biomarker' responses (at the individual level) to effects at population and ecosystem level.

To summarize: due to the broad definition of biomarkers used so far, there is a great overlap with currently accepted terms such as 'bioindicator' and 'ecological indicator'. In addition, the papers discussed above all implicitly or explicitly use their own definition of the term 'biomarker' and thus generate a number of slightly different interpretations. As a result, each of the authors gave a different answer to the question raised by the ESF. It is evident that there is a need for a clear definition of biomarkers.

## Early warning and ecosystem effects

From its introduction, the biomarker concept has been associated with an 'early warning' of pollution-induced stress. This early warning may have two meanings. First, it may indicate the detection of pollutant effects early in time, e.g. shortly after emission has started, the warning being that effects may develop with time at higher levels of biological organization (e.g. populations and ecosystems). Second, it may indicate the detection of a response at concentrations below those causing irreversible effects. In this case, the warning is that organisms are stressed, but it does not necessarily mean that more severe effects will develop with time. When 'early warning' and biomarkers are considered to be linked, it automatically implies that both concepts deal with situations of sublethal exposure. Conversely, the 'early warning' and biomarker concepts have not been developed to deal with situations of acute exposure, where entire trophic levels are impacted upon.

The dualistic meaning of 'early warning' mentioned above is one of the problems we stumble across when we want to answer the question posed by the ESF 'to what extent biomarkers can be used in the assessment of environmental "damage" and in the formulation of regulations to control such damage'. In our view, biomarkers can tell us that there is a deviation from health at the individual level, but whether this will result in effects at higher levels of biological organization, remains to be seen.

## Biomarkers, bioindicators and ecological indicators

To structure the discussion, we suggest the use of definitions that are linked to the different levels of biological organization presently used in ecological effects assessment. We propose the following definitions.

(1) Biomarker: any biological response to an environmental chemical at the below-individual level, measured inside an organism or in its products (urine, faeces, hairs, feathers, etc.), indicating a departure from the normal status, that cannot be detected from the intact organism. Thus, we want to restrict the term biomarker to biochemical, physiological, histological and morphological (including appearance, pigmentation, surface deformation, etc.) measurements of 'health' and exclude behavioural effects.

(2) Bioindicator: an organism giving information on the environmental conditions of its habitat by its presence or absence and its behaviour. Effects at, for example, the physiological level are not included in our definition of bioindicator.

(3) Ecological indicators: parameters describing the structure and functioning of ecosystems. In fact, this is limited to the ecosystem parameters also mentioned by Cairns and McCormick (1992), e.g. species diversity, population dynamics and nutrient cycling rates.

The advantage of the above definitions is that a clear distinction is made between responses at different levels of biological organization. These definitions are exclusive as overlap between the different terms is avoided. The biomarker concept defined here closely agrees to the more restricted definitions given by others (e.g. NRC 1987, McCarthy and Shugart 1990, Ernst and Peterson 1994).

Biomarkers are intended for use as instruments for environmental assessment in the field, but have, of course, been developed in the laboratory. The use of biomarkers in toxicity tests, as suggested by Depledge and Fossi (1994), can be viewed as an attempt to link biomarker responses to effects on life-history characteristics (such as survival and reproduction), which will give a further foundation to the use of biomarkers in environmental assessment. It is true that some experimental laboratory techniques, which are also used in biomarkers, may give 'a holistic picture of toxicity' or may help us to 'understand the mode of action of chemicals'. In such cases of laboratory research, we urge not using the term biomarker but restrict this term to the environmental assessment instruments.

The bioindicator definition given is based on the definition given by Sheehan *et al.* (1984): 'In its pure form the indicator concept states that the continued presence of certain species is an indication of the existence of a unique set of acceptable environmental conditions, whereas its absence would indicate the lack of appropriate environmental conditions.' In our definition, the behaviour of the indicator is included. Contrary to the definitions given by Hunsaker (1993) and Adams (1990), our bioindicator definition clearly excludes biomarker measurements.

As a consequence of these definitions, mussel filtration activity in the field has to be considered a bioindicator rather than a biomarker, whereas the numbers of snails showing imposex and pollutant-induced asymmetry are classified as biomarkers.

Chemical residue levels in organisms from the field may give an indication of exposure, but they cannot be considered as biomarkers or bioindicators as they do not provide an indication of deviations from 'health'. In many cases, residue levels of chemicals in organisms are the result of a passive process (equilibrium partitioning). Although it is not yet known what the impact of most metabolites is, metabolite levels in an organism or in its excreta are the result of a clear interaction of a chemical with the biological matrix and often reflect the induction of enzymatic reactions. Metabolite levels could therefore be considered biomarkers.

Effects on the genetic make-up of organisms seem hard to classify under the given definitions. Such effects may lead to changes at higher levels of biological organization, which are measured as bioindicators (e.g. species abundance) or ecological indicators (e.g. species diversity). Modern DNA fingerprinting techniques using large numbers of individual organisms to determine the genetic make-up of a population should be classified as biomarkers. Similarly, detection of DNA adducts, formed upon exposure to mutagenic and carcinogenic chemicals in the field, must be considered as biomarkers.

## Assessment of ecosystem effects

A major challenge of ecotoxicology is to predict effects at the population and community level from effects at the individual level. In our opinion, the sequence described in Table 1 should be followed for a stepwise ecological risk assessment of chemicals. Four different levels can be identified, at which effects of chemicals may become apparent.

(1) At the level of biochemical and physiological processes, deviations from the normal situation ('health') can be measured using biochemical techniques and these can be named biomarkers. Prediction of effects at the individual level using biomarkers is difficult, as often relationships between biomarker responses and survival, growth or reproduction effects have not yet been established (Depledge and Fossi 1994, Lagadic *et al.* 1994). A proper understanding of these relationships is a prerequisite for the use of biomarkers in ecological risk assessment.

(2) Survival, growth and reproduction of individuals are chosen as the end-points of the classic laboratory ecotoxicity tests used in the evaluation of new chemical substances and pesticides. These parameters are considered to be the most relevant characteristics for predicting effects at the population level and are also used in bioassays to assess the 'quality' or potential risk of environmental samples applying similar laboratory test methods.

(3) At the level of populations, effects become manifest as changes in the genetic structure, the age structure or the abundance of a population. Demographic studies are needed to detect effects at the population level. Such effects may be predicted from a thorough knowledge of the life history of an organism and of the effects of the chemical on the life-history characteristics (Van Straalen *et al.* 1989, Begon *et al.* 1990). Population dynamics theory may be helpful in the translation of effects from the individual level to the population level (Calow 1994).

(4) At the level of communities and ecosystems, changes in species composition, species abundance and diversity may occur. As a consequence of, for example species interactions, such effects cannot yet be accurately predicted from effects at the population level, as was recognized by Lagadic *et al.* (1994) and many others (e.g. Adams 1990, Forbes and Forbes 1993, Hopkin 1993, Smith and Cairns 1993, Van Straalen and Løkke 1996).

Following this reasoning, the effects of toxicants on ecosystem health cannot yet be predicted accurately from the end-points measured in laboratory toxicity tests. Similarly, biomarkers do not yet allow a proper assessment of ecological effects (Calow 1994). This is not recognized by Depledge and Fossi (1994), who described a protocol for the use of biomarkers in ecological risk assessment. Besides the extrapolation from biomarker responses to effects at the individual level discussed above, their approach

**Table 1.** Framework for the linkage between biomarker response and ecological effects after exposure to (a) chemical pollutant(s), and preferred working definitions

| | Effects at the level of: | | | |
|---|---|---|---|---|
| | Biochemical and physiological processes | Individual organisms (species) | Populations | Community and ecosystems |
| Endpoints | Enzyme activity, stress proteins, DNA adducts, etc. | Survival, reproduction, growth, behaviour | Age structure, abundance | Species composition, diversity, abundance |
| How to measure? | Biochemical methods | Laboratory toxicity tests | Demography/prediction from laboratory toxicity tests using life-history data | Complete analysis of community structure and ecosystem processes |
| Preferred term | Biomarker | Ecotoxicity test, bioassay | Bioindicator | Ecological indicator |

involved the selection of important populations and the use of key species and sensitive species. We have doubts whether it will be possible to identify key species. Usually, there is a number of species which together take care of a certain process or function. These species can be identified as a functional group (Faber 1991, De Ruiter *et al.* 1994). Some functional groups consist of only a small number of species and, therefore, the process involved might be more susceptible to disturbance. Soil nitrification, for example, is often viewed as such a process. Earthworms, as another example, have a special position in the soil ecosystem, playing a key role in litter decomposition and the development of soil structure. As to sensitive species, the question has to be raised: sensitive to what? There is no such thing as a most-sensitive species (Cairns 1986), as sensitivity will strongly depend on the chemical involved and the intrinsic properties of the organism (Crommentuijn *et al.* 1994).

We would strongly recommend the implementation of modern ecological techniques in ecotoxicology. Recently, Van Straalen and Løkke (1996) elaborated on the use of molecular techniques for the analysis of population structure, reaction norms (phenotypic plasticity) in life-history evolution and food-web theory to analyse community structure and stability as potential ecological concepts to be applied in ecotoxicology.

It should be remarked that biomarkers can only be used as tools in retrospective risk assessment (Suter in McCarthy and Shugart 1990, Hunsaker 1993), since a biomarker response indicates that there has already been an exposure. Modern ecotoxicology, with the biomarker concept, offers diagnostic techniques which allow for an identification of chemical stressors. This approach is advocated by Cormier and Daniel (1994), who recommended the use of biomarker techniques in combination with community measurements to determine 'real-time status of organism health'.

## The future of biomarkers in ecological risk assessment

Most of the biomarkers discussed here respond specifically to a (limited) number of well-known (groups of related) chemicals. Peakall and Walker (1994) gave an overview of a number of such specific and less-specific biomarkers. We believe that many environmental problems are only partly related to these well-known pollutants for which we have specific biomarkers. Ecosystems might also be affected by other chemicals or mixtures of known (and unknown) chemicals. We realize that among the well-known chemicals are a number of pesticides, which are developed to have an effect and for which it is fairly easy to develop a specific biomarker. Still, it would be desirable to also have biomarkers indicating health effects of other, less well-known chemicals. Further research on such general biomarkers is required (Ernst and Peterson 1994, Lagadic *et al.* 1994).

To allow for a proper health assessment, we agree with other authors (e.g. Benson and Di Giulio 1992, Depledge and Fossi 1994, Ernst and Peterson 1994) that it is essential to know what are the normal levels of a biomarker, how they may be affected by biotic (age, sex, moulting cycle, adaptation, interindividual variation, etc.) and abiotic conditions (temperature, season, pH, soil moisture, salinity, etc.) and how persistent the biomarker response is with time. For chemical-specific biomarkers, knowledge of the biochemical pathways of that pollutant and its interactions with biochemical processes in the body is required to relate a change in health to pollutant exposure.

## Conclusions

(1) Biomarkers, bioindicators and ecological indicators are useful descriptors of field situations which may allow the identification of chemical stressors and their potential ecological risk. They give additional information that cannot be obtained from chemical analysis of pollutant concentrations alone, and they may integrate effects of mixtures of chemicals over long exposure periods.

(2) Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms: a 'biomarker' is defined as any biological response to an environmental chemical at the below-individual level, measured inside an organism or in its products (urine, faeces, hairs, feathers, etc.), indicating a departure from the normal status, that cannot be detected from the intact organism. The term 'bioindicator' should be restricted to an organism giving information on the environmental conditions of its habitat by its presence or absence or its behaviour. The term 'ecological indicator' is equivalent to the term ecosystem parameter, describing the structure and functioning of ecosystems.

(3) To answer the questions raised by the ESF: biomarkers are designed to assess, at an early stage, 'damage' at the below-individual level. With the present knowledge, it is however, not yet possible to extrapolate effects on individual organisms to the level of populations, communities and/or ecosystems. Biomarkers can, therefore, not yet be used to assess ecological damage. To allow for a sensible use of biomarkers in ecological risk assessment, the relation between biomarker response and exposure levels, on one hand and effects on life-history parameters, on the other should be determined.

(4) The implementation of modern ecological techniques in ecotoxicology is recommended as an approach to bridge the gap between individual response and ecosystem effects.

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