Polish Journal of Occupational Medicine and Environmental Health, Vol. 5, No 2,119-128, 1992

REVIEW PAPERS

THE ROLE OF BIOLOGICAL MARKERS IN TOXICOLOGY

JANUSZ HANKE and JANUSZ A. INDULSKI

The Nofer Institute of Occupational Medicine, Lodz, Poland

Key words: Exposure, Effect, Susceptibility, Application of biomarkers

Abstract. Biological markers are tools that can be used to clarify the relationship, between exposure to a xenobiotic compound and health impairment and to indicate individual or population differences that affect the biologically effective dose. Biological markers, broadly defined, are indicators of variation in cellular or biochemical components or processes, structure, or function that are measurable in biological systems or samples. There is growing interest in the use of biological markers to study the health effects of exposure to environmental toxicants in occupational medicine, epidemiology, toxicology, and related biomedical fields.

I. CONCEPTS AND DEFINITIONS

Introduction

The scientific revolution is encouraging the identification of biological markers, which are thought to offer great promise in reducing the uncertainties associated with toxicology. "Biomarkers" as defined by the National Academy of Sciences (12), are indicators of variation in cellular or physiological components or processes, structures or functions that are measurable in biological systems or samples.

Understanding the human health risk associated with environmental exposures involves defining the cascade of events between exposure to an environmental agent and the resulting health effect. To have an effect on health, a pollutant or its metabolite must be absorbed into the body, reach a target organ, and result in a biological change (13).

According to the classical approach, one may distinguish three general categories of biological markers, those of exposure, those of effect, and those of susceptibility.

A biological marker of exposure is an exogenous substance or its metabolite or

Address reprint requests to J. Hanke, Department of Laboratory Diagnostics, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment with an organism. A biological marker of effect is a measurable alteration of an endogenous component within an organism that, depending on its magnitude, can be recognized as a potential or established health impairment or disease. A biological marker of susceptibility is an indicator of an inherent or acquired limitation of an organism to respond to the challenge of exposure to a specific xenobiotic substance.

From toxicological practice it is well known, that such a division of biological markers simplifies reality. The classical conceptions of biological markers are not uniform. Some examples of different meanings are included in Table 1 (exposure) and Table 2 (effect).

Although scientists tend to divide biological markers into groups, it seems evident that there is a continuum between health and disease, and advances in toxicology have demonstrated a continuum between exposure and effect.

Table 1. The ambiguity of the term "exposure".

External exposure is the amount of concentration of xenobiotic material in the environment of an organism.
Internal dose is the amount of xenobiotic material that is transferred to or absorbed into the organism.
Biologically effective dose is the internal dose that is quantitatively correlated with identifiable biological effects; however, it is considered to be the amount of xenobiotic material that has interacted with a critical cellular or tissue receptor or target where the biological effect is initiated.
Surrogate size Because the receptor sites are often not known, or are not accessible for sampling, it is frequently necessary to use a surrogate site for which the dose has been correlated with the biologically effective dose or the indentifiable biologic effect on the target.
Body burden is the total internal dose or that which has accumulated over time in the organism. Depending on the toxokinetics of the agent, body burden might be a biological marker of exposures that have occurred recently or in the distant past. Body burden includes the dose at the target receptor sites, but it can also include amounts of xenobiotic material stored in other, nontarget compartments.

Table 2. The ambiguity of the term "effect".

An alteration in the tissue or organ.
An early event in a biological process that predicts the development of health impairment.
A health impairment or clinically recognized disease.
A response, peripheral or parallel, to a disease process, but correlated with it and thus, useful in predicting the development of health impairment.

Biological markers of exposure reflect in certain conditions something that already represents an effect and vice versa. Similarly it happens with biological markers of susceptibility.

Carboxyhaemoglobin (COHb), free erythrocyte protoporphyrin (FEP) and delta-aminolevulinic acid can serve as an example of a term which is situated in the borderland between the biological markers of exposure and biological markers of effect.

Carboxyhaemoglobin concentration after exposure to carbon monoxide has been used to indicate internal dose and to predict effects. Blood lead content might appear to be a more direct indicator of internal lead dose than a lead-induced increase in free erythrocyte protoporphyrin and delta-aminolevulinic acid. In some clinical situations, however, FEP is a more valid marker of the total body lead burden than is the lead content. That might also provide more accurate information about the biologically effective dose of lead in target organs, such as the brain (8). It has been well established that the more recent exposure to some chemicals can modify the susceptibility (e.g. sensitization to formaldehyde) or, similarly, a pre--existing disease state, that increases the internal dose, or a biologically effective dose amplifying the effect on the target tissue (15, 18).

Surrogate size

If a biologically effective dose is correlated with an effect or concentration at a peripheral site, this can function usually as a surrogate for the dose or effect that is occurring in the target tissue. Because the receptor sites are often not known, or are not accessible for sampling, it is frequently necessary to use a surrogate site for which the dose has been correlated with the biologically effective dose (or the identifiable biological effect) on the target. Such surrogates can be used as markers of exposure and effect on the site of action.

The measurement of red blood cell delta-aminolevulinic dehydratase (ALAD), an enzymatic marker of the biological effect of lead (6), which is probably not participating in the synthesis of hemoglobin can be given as an example of surrogate compartments. An example of a marker that is closely related to the external dose, the biologically effective dose, and health status, is the use of lymphocyte DNA adducts. These are used as markers of absorbed dose at the molecular site of action, and can indicate the likelihood of cancer (19). Although the presence of lymphocyte DNA adducts of mutagenic alkylating xenobiotic compounds reflects a biochemical effect, they may also be considered as markers of biologically effective doses of carcinogens that are evenly distributed. Unless the lymphocyte itself is the precursor of a tumor, the whitecell DNA adducts are appropriately considered to be indirect markers of a biologically effective dose in the target organ (15).

Principles of selecting markers

The proper selection of biological markers is not an easy problem. Biological markers of exposure can be obtained by measuring the concentration of a particular toxicant or its metabolites alone or those connected with DNA, RNA, proteins or receptors in tissue or fluids, obtained in *vivo* or *in vitro*. The use of markers can be

complemented by questionnaires. Markers of effect can be obtained by biochemical analyses of organspecific events.

The use of biological markers to screen the human population involves marginally invasive techniques. Many techniques well adapted to laboratory animals cannot be readily applied to the human population. The use of biologic markers should also involve procedures that are readily acceptable by the subjects.

Once a biomarker is developed, it must be validated. The validation process will be more fully described below.

It might be reasonable to use a battery of markers, because limitations are associated with most of them. For example, to determine whether there is a potential or actual adverse effect on male reproductive function caused by the exposure, a battery of markers might be tried (Table 3). Although each of the tests has inherent limitations of specificity and sensitivity with varying predictive values, a battery tests can be a powerful tool for identifying the dysfunction.

Table 3. A battery of biological markers.

1. Concentration of the toxicant in urine or in seminal fluid.
2. Sperm density in ejaculation.
3. Sperm morphology (seminal cytology),
4. Interspecies sperm penetration assay (as an indicator of sperm chromosomal abberations).
5. Determination of the plasma concentration in hormones (e.g. testosterone).

Ethical issues

The use of biological markers has raised a number of important ethical issues (2). These are related particularly to markers of susceptibility. Social factors have appeared as exceptionally important. In 1981 in a fourpart series in *The New York Times*, the issue of genetic screening of individuals prior to entry into the workplace of certain industries was discussed (20). According to the opinions presented, the policy was highly controversial, with numerous social, legal, medical and economic implications. As a result of *The New York Times* series *The Genetic Testing and Employment Act* was proposed to stop the genetic screening of workers in New York. The following arguments supported the Act:

1. The notion that employees have weak or defective genes is an unfair invasion of the individual's privacy;
2. The hazard, not the workers, should be removed from the workplace; and
3. There is a strong likelihood that a screening programme may be a subtle form of racial discrimination.

The biological problems associated with job preplacement examinations have been studied by Motulsky (11). According to the authors the idea concerning the use of tests involving innate individual characteristics of workers, however, apparently useful in the job assignment and reduction of unnecesary health risks, should not be promoted. It is important to distinguish between markers that totally predict an adverse effect, reasonably predict, or only minimally predict. A situation may develop in which a harmless deficit of an enzyme or a protein could be used as an argument for discrimination against racial or ethnic groups with high frequencies of such deficits (5).

II. VALIDATION OF BIOLOGICAL MARKERS

Once a biomarker is developed, and is to be used in human study it must be validated. The process of validation is a complex one. Its individual stages are discussed below.

The confirmation of credibility of the biological marker

To validate the use of a biological measurement as a marker, it is necessary to understand the relationship between the marker and the event or condition in question. In validating biological markers, animal models are useful for understanding the mechanical bases of the expression of markers and relationships among exposure, early effects, and disease. A useful approach to the validation of marker data is to conduct experimental studies in animals or in materials *in vitro*.

Sensitivity and specificity

Sensitivity and specificity are critical components in the process of validation (9). Sensitivity is the quality of an epidemiological test method that is able to identify correctly those who have the disease or condition in question.

Specificity relates to identifying correctly those who do not have the disease or condition in question.

A major purpose of markers in environmental health research is to identify vulnerable persons, so that, risk can be predicted and disease prevented.

The identification of the strength of biological plausibility

Particularly critical for markers is the strength of biological plausibility that allows the association between a change in a specified signal and the occurrence of a specific exposure or a change in the probability of a specific outcome. The goal is to develop markers that reliably indicate an early stage in the development of a disease in humans, when effective intervention is still possible. If a disease can be satisfactorily induced in experimental animals, then potential biological markers for predicting eventual disease can be explored, and early indicators of the disease may be identified for use in epidemiological studies. Biological markers observed well before the onset of desease might have low predictive value for the disease itself, nevertheless, may function well as criteria for defining exposed populations.

Validation of forward and backward processes

Validation involves both forward and backward processes of association — i.e. from the marker backward to exposure and from marker forward to effect. A complete understanding of the limitations of a given biological marker is crucial for its application and interpretation.

The expected uses

Validation of a specific marker also depends on its expected use. Biological markers could be actually recommended for assessing the exposure and the effect. Ideally, the tests selected for detecting the occurrence of xenobiotic exposure, and resulting effects are sensitive, specific, inexpensive, minimally invasive, attended by low risk, and easily used in a large population. Not all biological markers are relevant to therapeutic intervention. Some may identify the exposure or presence of disease but not provide information relevant to a therapeutic decision. Thus, in some circumstances, the diseased or otherwise affected person would not benefit from the information yielded but would function as a biological marker for the community, allowing public health measures to prevent further exposure or disease.

The analysis of cost-effectiveness

After discussing some theoretical problems we are now approaching certain practical questions related to the biological markers validation. In some cases, cost-effectiveness is also a factor, particularly for clinical measurements intended to be used for screening, rather than research.

Extrapolation from animals to humans

Extrapolation to humans should be based on the most sensitive animal species, barring clear evidence that the species is toxicologically distinct from humans. Laboratory animals and humans can differ in toxicokinetics. Thus, the use of animal data to determine health risk in humans must be assessed carefully, especially when the data are derived from the monitoring of external exposure. The toxicity of some chemicals is mediated either by activation or by detoxification biotransformation reactions. In as much as biotransformation differs among species, it is important to establish whether the routes and rates of human and animal metabolic pathways are similar. A useful approach to the validation of marker data is to conduct experimental studies in animals and clinical studies in humans to develop information that would allow the comparison of the interspecies (10). During the past years, the EPA has issued guidelines for extrapolation from animals to humans (23). The development of biological markers might enable scientists to make better use of laboratory animal data in estimating the effects of chemicals in humans.

Quality assurance

Quality assurance and quality control are fundamental in the objective development and application of accurate and verifiable biological markers. The objective of laboratory quality assurance practices is to ensure that findings reported by one laboratory are, in fact, verifiable and fall within acceptable limits of a measurement error.

The FDA has developed a set of guidelines known as *Good Laboratory Practices* (22), which are now incorporated into the standard procedures of most testing and

analytic laboratories. The preparation of biological standards for measuring markers of effects is rather complex. Cell-culture systems might provide particular types of standards; in some cases the chemicals that constitute the marker (metabolic product, intermediate or other material, such as protoporphyrin) can be synthesized and incorporated into the appropriate biological matrix to meet the criteria of quality assurance.

Application of GLLPs in the analysis of biological samples, especially human tissue, has been reviewed by operational units of the Center for Disease Control, The National Bureau of Standards, and various clinical laboratories (1, 13, 14).

III. APPLICATION OF BIOLOGICAL MARKERS

Occupational medicine

In occupational medicine, answers to two questions are frequently required:
- Who should be prevented from being exposed?
- Who should be immediately withdrawn from exposure?

The obtaining of the right answers to the posed questions is potentially feasable using the biological markers.

The biological markers of susceptibility indicate the individual or population factors which can affect the response to environmental agents. Susceptibility markers are indicators of whether the individual or the subpopulation is more or less sensitive to exposure to a particular enviromental pollutant. A sensitive subpopulation, for example, can be pinpointed by an increased absorption rate or by a more severe biological response. According to Stockinger and Scheel (21), authors of a consensus report, the prerequisites for a hypersusceptibility test in industry must fulfil the following conditions:

1. The abnormality or deficit must exhibit a relatively high prevalence in the working population;
2. The test must concern substances commonly occurring in industry;
3. The condition must be compatible with an apparently normal life before the onset of industrial exposure.

Using the biological markers of susceptibility in a screening of individuals prior to entry into the workplace (job preplacement examination) enables the assignment of a proper job to the individual characteristics of the candidate and, thus, reduces unnecessary health risks.

As was said above the use of biological markers of susceptibility has raised a number of important ethical issues (25). Can an employee be forced to leave his job once a susceptibility marker has been detected? Is it important to distinguish between markers that are totally predictive, or only minimally predictive?

In assessing the predictive value of biological markers, it is necessary to account for the heterogeneity of the human population which is composed of persons who differ in age, genetic constitution, nutritional state and general health. It is also necessary to identify persons who are likely to exhibit the earliest and most severe

effects of exposure. Markers of early biological effect include alteration in the function of target tissue after exposure. As early warning signals, such markers can be useful dosimeters to guide intervention aimed at reducing or preventing further exposure. Biological markers of altered function are most useful if related to a specific organ – e.g. beta-microglobuline for kidney function.

Use of biological markers in risk assessment

Cellular and molecular markers can be powerful tools in assessing the risk associated with exposure to environmental toxicants. The biological marker of exposure or of effect can be useful in hazard identification – e.g. it is a qualitative step by which the environmental agent is causually associated with an adverse effect. The use of biological markers, indicating exposure and dose in molecular epidemiological studies, provides an opportunity to determine the shape of the response curve in humans. A common source of uncertainty in the risk assessment is a dose-response relationship at low doses or for rare effects (16, 17). It is often impractical to conduct studies of effects at low doses, because large numbers of animals are required to detect a low incidence of effects (24). Demonstrable health effects in human, given the limits of epidemiology, are often associated with high doses, and hence high risk. Sensitive molecular markers being developed will permit the study of relationships between exposure to chemicals at a low ambient concentration of a molecualr marker predictive to human risk.

The biological markers in epidemiology

Biological markers function well as criteria for defining the exposed population and thus, are useful in a long-term follow up. Biological markers can also be used to determine a dose-response relationship, especially at the low doses relevant to the exposure to most environmental chemicals. A tissue affected by the toxicant might exhibit an altered function even if the exposed person has no overt manifestations. Such an altered function can be, in some cases, determined by testing, particularly with biochemical methods. In 1977 Higginson (7) described molecular epidemiology and the use of markers as the application of sophisticated techniques to the epidemiological study in biological material. Perera and Weinstein (19) later defined molecular cancer epidemiology as an approach combining the analytic epidemiology and biochemical or molecular techniques in order to identify the role of exogenous agents or host factors in the generation of human cancer. Epidemiology uses markers as indicators of internal dose or of health effects.

Ecological biomarkers

Ecological biomarkers are biological responses to anthropogenic agents which occur within an organism (4). Ecological biomarkers are cellular, biochemical and physiological responses of the humans and animals to antropogenic agents such as toxicants, utlraviolet radiation, and conventional pollutants. Biomarkers can be used in a systematic fashion to diagnose the cause of an identified defect in a wild

population of an organism collected from the environment after a prolonged and complicated toxicant exposure history.

It is clear that there is both a legislative and pragmatic need for the use of tools for assessing the effect of pollutants in the open environment. With thousands of new chemicals and other antropogenic agents which are entering the environment, it is imperative to have the capability to identify and monitor the causative agent of environmental effects and sensitive indicators of those effects in free ranging animals and wild plants.

Ecological biomarkers can provide a glimpse of the health and exposure of an organism which lives in the wild. This is a clear advantage over laboratory toxicity testing, which provides only limited levels of pollutant exposure. Since a biomarker can be used to diagnose the health and exposure of organisms in the wild, it is tempting to use biomarkers to predict the present and future status of a population of wild organisms.

REFERENCES

1. ACS (American Chemical Society), Committe on Environmental Improvement and Subcommittee on Environmental Analytical Chemistry. Guidelines for data acquisition and data quality evaluation in environmental chemistry. Anal Chem 52: 2242–2249, 1980.
2. Ashford NA, Spadafor CJ, Caldart CC. Human monitoring. Scientific, legal and ethical considerations. Harvard Environ Law Rev 8: 263–363, 1984.
3. Fowle JR, Collins E. ORD Health biomarkers program Research Strategy Document. Office of Research and Development. US Environmental Protection Agency, Washington DC. 1–23, 1991.
4. Garet Levis, Ecological Biomarkers: Application and Research Office of Environmental Process and Effects Research. Office of Research and Development. US Environmental Protection Agency, Washington DC. 1–13, 1991.
5. Hanke J, Indulski J. Genetic screening – An absolute necessity or a misconception? Pol J Occup Med 1: 39–44, 1988.
6. Hernberg S. Biochemical and clinical effects and responses as indicated by blood concentration, In: Lead Toxicity. Eds RL Singhal, JA Thomas, Urban and Schwarzenberg, Baltimore, 367–399, 1980.
7. Higginson (1977) after: Report of the Oversight Committee. Biologic Markers in Reproductive Toxicology. National Research Council. National Academy Press. Washington DC, 396, 1989.
8. Lauwerys RR. Industrial Chemical Exposure: Guidelines for Biological Monitoring. Davis, Calif. Biomedical Publications, 150, 1983.
9. MaMahon B, Pugh TF. Epidemiology: Principles and Methods. Little, Brown and Co. Boston, 376, 1970.
10. McClellan RO. Health effects of diesel exhaust: A case study in risk assessment. Am Ind Hyg Assoc J 47: 1–13, 1986.
11. Motulsky AG. Bioethical problems in pharmacogenetics and ecogenetics. Human Genetic Suppl 1: 185–192, 1978.
12. NAS (National Academy of Sciences), Biologic markers in reproductive toxicology. National Academy Press, Washington DC, 395, 1989.
13. NCCLS (National Committee for Clinical Laboratory Standards). List of Standards. Villanova, Penn. National Committee for Clinical Laboratory Standards, 1981.
14. NCCLS. List of Standards. Villanova, Penn. National Committee for Clinical Laboratory Standards, 1985.
15. NIEHS (National Institute for Environmental Health Sciences) Task Force 3. Biochemical and

Cellular Markers of Chemical Exposure and Preclinical Indicators of Disease. Washington, DC, US Department of Health and Human Services, 1985.
16. NRC (National Research Council), Risk Assessment in the Federal Government: Managing the Process. Washington, DC. National Academy Press, 191, 1983.
17. NRC, Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects, Washington, DC. National Academy Press. 337, 1986.
18. Omenn GS. Susceptibility to occupational and environmental exposure to chemicals. Prog Clin Biol Res 214: 527–545, 1986.
19. Perera FP, Weinstein IB. Molecular epidemiology and carcinogen – DNA adducts detection. New approaches to studies of human cancer causation. J Chronic Dis 35: 581–600, 1982.
20. Severo R. The genetic barrier: job benefit or job bias? The New York Times, 3–6, February, 1980.
21. Stockinger HE, Scheel LD. Hypersusceptibility and genetic problems in occupational medicine. A consensus report. J Occup Med 15: 564–573, 1982.
22. US FDA (United States Food and Drug Administration), Good laboratory practice for nonclinical laboratory studies. Code of Federal Regulations. Title 21, Chap. 1. Pt. 58, Washington DC, US Governmental Printing Office, 1988.
23. US EPA (United States Environmental Protection Agency), Health effects testing. Reproduction and fertility effects. Code of Federal Regulations. Title 40, Chap. 1, Pt 798, section 4700, Washington DC, US Goverment Printing Office, 1987.
24. Wilkinson CF. Being more realistic about chemical carcinogenesis. Environ Sci Technol 21: 843–847, 1987.
25. Yodaiken RE. Surveillance, monitoring, and regulatory concerns. J Occup Med 28: 569–571, 1986.

Received for publication: March 5, 1992
Accepted for publication: April 27, 1992