Early detection of hazardous exposures of humans or an ecosystem may significantly reduce adverse effects through appropriate reductions in the presence of those chemicals in the general environment and/or occupational settings. The adverse effects of concern for humans are depicted in Figure 4.1. For non-human targets, the concern is the preservation of the stability of populations and of ecosystems including agroecosystems, and forests. General references to health and health risk include the health of ecosystems and their constituents (Figure 4.1).

Currently there are many indicators that may be used for the identification of exposure to possibly hazardous chemicals (Figure 4.1). This chapter deals primarily with those methods that measure internal exposure (qualitative determination of the presence of chemicals in the organism) or internal dose (quantitative determination of the amount of chemicals in the organism). Biological exposure markers as measured with available methods can be divided into markers based on some biological effect (e.g., cholinesterase inhibition by organophosphorus compounds) or on the determination of a chemical or its metabolite(s). Biological exposure markers do not necessarily require a relation with any of the health effects of concern. Levels in the environment (concentration in a water column, for example) and chemical residue levels in an organism, are the most widely used methods for ecotoxicology. Additionally, ecosystem responses and biological markers can be used to determine exposure.

Although the methodology to measure internal exposure and dose in the organism is still in its infancy (Lohman et al., 1984; Baan et al., 1985), an increasing number of sensitive physical, chemical and biological methods are currently available. The advantages and disadvantages of biological monitoring compared to traditional chemical analysis of the environment are considered elsewhere. All methods available measure internal exposure or internal dose in either a direct (chemical-specific) way or indirect (chemical-dependent biological effect) way. In some cases, both direct and indirect methods demonstrate an alteration of a normal biological process,
and these alterations may retrospectively or mechanistically be related to the adverse effects of concern. As a result, some tests, especially those with biological endpoints, are “earmarked” as indicators for early health effects. Whether such a generalisation is justified seems doubtful; this chapter will demonstrate that, for each chemical or group of chemicals, calibration of the health significance of the biological effect is required before a test can be used as an indicator of an adverse effect of concern. In practice, however, premature “earmarking” of a test as an indicator of early health effects automatically removes these tests from the “pool” of tests to be used for measuring internal exposure or dose; the results obtained with such tests will be interpreted subjectively.

In this approach, methods should not be earmarked too soon. Preferably, the performance of all available methods in a tier (decision-tree) model should be analysed as depicted in Figure 4.2. The methods are first screened for their sensitivity and specificity to detect exposure or dose of a chemical before test results are considered as a biological marker for the injury of interest. Although, in practical situations, no methods are applied before incurring suspicion about the hazardous effect of a chemical (Figure 4.1), the decision-tree model in Figure 4.2 makes obvious that many actions can
be taken on the basis of exposure measurements before the results of a test are earmarked (through calibration) as an indicator of possible adverse health effects.

The approach described herein requires an alteration of existing operational definitions. In a seminar organised by EEC, NIOSH and OSHA (for review see Zielhuis, 1985), suggested operational definitions have included:

1. Ambient (environmental) monitoring (EM)
   the measurement and assessment of agents at the place of concern to evaluate ambient exposure and health risk compared to an appropriate reference.

2. Biological monitoring (BM)
   the measurement and assessment of agents or their metabolites either in tissues, secreta, excreta or any combination of these to evaluate exposure and health risk compared to an appropriate reference.
In the approach depicted in the decision-tree model of Figure 4.2, these operational definitions must be changed in such a way that the words “and health risks” have to be replaced by “or health risks.” This allows analysis of the performance of available tests in relation to their use; i.e., in tests to measure internal exposure/dose, the sensitivity, specificity, and background fluctuation; in tests to measure a biological effect marker, the relevance of the marker for the adverse effect of interest.

After internal exposure has been identified, consideration will be needed to determine whether the endpoint(s) measured in the test may also be indicative for some adverse health effect in an exposed population. Such a correlation does not need to be causal; it would be sufficient if only a correlation can be found between the test endpoint and an adverse effect in animals. Any tests (physical, chemical or biological) may be of value; the only requirement is the availability of an appropriate model for calibration.

At this point, health surveillance (HS) becomes meaningful. HS is operationally defined as the periodic physiological examination of exposed workers to prevent occupationally-related diseases. Because health surveillance is related to disease, a calibration process is obligatory before a biological marker can be used for this purpose.

Most current methods do not allow the measurement of target cell exposure, especially not in humans. For certain endpoints of concern like carcinogenicity or mutagenicity, methods are under development that may allow target dose determination (e.g., protein alkylation or DNA adduct formation), especially in those cases where a predictive relation exists between target dose and internal dose to agents such as radiation or ethylene oxide.

The methods described are still of limited use, especially for risk estimation. This is due not only to the intrinsic properties of the tests (i.e., specificity, sensitivity, etc.) but also to the fact that, in practice, humans and the ecosystem are exposed to complex mixtures of potentially hazardous chemicals; and, as depicted in Figure 4.3, the penetration and metabolism of chemicals in organisms is quite complicated, and differs widely among members of a population and within individuals (see Yashin et al., this volume). Furthermore, the mechanistic relation between a biological marker and an adverse health effect is in most cases only poorly understood.

4.1 TOXICOKINETICS

The prerequisites of biological monitoring are the following conditions:

(1) A chemical substance and/or its metabolite(s) are present in some tissue(s), body fluid(s), or excreta suitable for sampling; in some instances,
monitoring of specific effects may help to identify exposure to a group of substances;
(2) Practical methods of analysis are available;
(3) The measurement strategy should be adequate (i.e., the samples must be representative);
(4) The result should be properly interpreted with established scientific principles; and
(5) Both ethical and legal requirements are met.

4.1.1 KINETIC CONSIDERATIONS

Before biological monitoring can be recommended as the method for exposure assessment, toxicokinetics and metabolism of chemicals of interest should be determined.

Some key toxicokinetic factors to be considered in monitoring human exposure are:
(1) Nature of the exposure (dose and dose interval/duration),
(2) Absorption efficiency,
(3) Rate coefficients for absorption and elimination and their possible variation with dose,
(4) Nature and availability of the toxicokinetic model (one, two, or multicompartment),
(5) First-pass effect and its magnitude (ingestion-liver, inhalation-lungs),
(6) Are metabolites the toxicologically active toxicants?
(7) How similar are metabolic pathways and rates for inhalation and ingestion?

For ecosystems, the transport between various compartments (water, sediment, biota) must be considered.

Toxicokinetic data provide a most useful tool for internal dose estimation. General conditions and assumptions are needed (Figure 4.3). First, the primary routes for human exposure are inhalation and ingestion; and data from animal studies using different routes of exposure are useful only when such factors as gastro-intestinal tract absorption efficiency, dose frequency, hepatic metabolism, and pulmonary absorption rates are known. Second, if the biological system manifests no clinically observed effects, the toxic chemical might be detoxified before reaching the target organ(s) or elimination rates might be sufficiently slow that blood levels fluctuate little during periodic dosing, provided that the portal of entry (e.g., liver or lungs) is not the target organ. Third, all factors that affect the concentration of the toxic compound at the target organ which may be different for each route of exposure are accounted for, including absorption efficiency and degree of activation or deactivation (i.e., the formation of more toxic metabolites and chemical breakdown and excretion).

For a relatively rapidly absorbed and slowly eliminated compound, dose interval and regimen influence very little the systemic concentration or temporal relationship; in such circumstances, it is the total amount rather than the kinetics of systemic uptake and elimination that are expected to influence systemic toxicity. The importance of the “first-pass effect” is very much dependent on the route of exposure. Substances absorbed via the lungs or injected intravenously are carried and distributed approximately equally to the vessel-rich organs of the body. However, substances administered orally pass from the gastro-intestinal tract via the portal system directly into the liver. Therefore, for substances that are subject to extensive hepatic metabolism, the effective dose reaching the systemic circulation is considerably reduced or totally eliminated. Similarly, compounds activated by the liver may have patterns of toxicity specific to their exposure route because of this first-pass effect.

Most chemicals are distributed in several different compartments of the body; some accumulate preferentially in specific tissues or organs. For example, lipid soluble compounds accumulate in the body fat, cadmium in the liver and kidneys, methyl mercury in the brain, and lead in the bones.

Another special case is the deposition of aerosols and particulates in the lung. Deposition varies with particle size; once deposition occurs, uptake
BIOLOGICAL MONITORING OF EXPOSURE TO CHEMICALS

of aerosols is remarkably similar, regardless of particle size, presumably due to their solubility. Certain dusts accumulate in the lungs with only limited distribution in other tissues. On the other hand, insoluble particulates of larger size may be carried up by the ciliary elevator and swallowed, possibly resulting in gastrointestinal absorption.

In biological monitoring, one aim is to estimate the uptake and/or internal dose of the chemical at a given time. An alternative approach is to determine the highest concentration attained in the body, especially in the target tissue, with which one can estimate the internal dose of a chemical. This is very much dependent on the chemical, especially its kinetic properties. For chemicals with a long half-life (such as lead, cadmium and mercury), the concentration in blood reaches a steady state which reflects the equilibrium between daily intake and excretion. For constant exposure, the daily variation in the concentration in blood and other body fluids is small, and an estimate of the internal dose can be obtained from even single determinations of blood or urine levels.

For chemicals with short half-lives, the approach is quite different. First, concentrations, especially in the blood, change rapidly with time, requiring standardisation of the specimen collection time. Fluctuations in ambient concentrations of such a chemical require frequent monitoring to obtain a representative estimate of internal dose.

In practice, it is not feasible to effectively monitor chemicals with a short half-life. However, for many chemicals, several successive half-lives can be obtained reflecting their distribution in different compartments of the body. It is important to note that lipid soluble organic solvents having a short half-life (of the order of minutes) in blood tend to accumulate in adipose tissues, and are slowly released after exposure has ceased. Fat deposits function as integrators, permitting estimation of exposure over time (i.e., several days) from specimens collected within a short period of time after cessation of exposure.

Most of the above applies to warm-blooded animals. For lower organisms, additional considerations exist. The first, and perhaps most obvious, consideration is the large difference that may exist among taxonomic strata in absorption, distribution, transformation, and excretion. These differences are related to the morphology, physiology, and metabolism of organisms. While it is clear that multicellular animals differ enormously from multicellular plants, and fish differ from amphibians, or insects differ from birds, closely related species may also handle a specified chemical quite differently, because of some relatively small genetic difference. When assessing the fate of chemicals, the life-cycle stage of an organism is also important. For example, insect eggs, larvae, immature stages, and adults will often respond quite differently to a chemical insult. So will dormant seeds react differently from growing seedlings. Toxicokinetic mechanisms are also strongly influenced in cold-blooded animals by environmental variables, particularly temperature which may greatly alter
uptake or metabolism of a chemical. Caution must be exercised in extrapolating exposure levels from concentrations in organisms.

The study of urinary excretion can also integrate exposure to chemicals. Thus, the measurement of metabolites in urine and the kinetics of their excretion are often preferable to measurement of original chemicals in blood.

Determination of the sites and the types of measurements depends on physico-chemical and biological properties of a given substance. The route of exposure often determines the tissues or organs in which a substance should be measured.

Concentrations of chemicals in blood or urine may not necessarily reflect its body burden. Several new approaches have been developed to estimate directly the amount of accumulated chemical at a site of deposition. The most widely used one is neutron-activation measurement of cadmium in liver and kidneys. Other methods gaining increasing interest are X-ray fluorescence of lead in bones and magnetopneumography of iron containing dusts in the lungs. These techniques have been reviewed by Lauwerys (1983) and Lippmann (1985).

4.1.2 TOXICOKINETICS AND EXPOSURE ASSESSMENT

How toxicokinetic data assist in estimating exposure is illustrated in Figure 4.3, which demonstrates the integration of external exposure, internal dose, early biological effects, and the biologically effective dose. By measuring concentration of toxic chemicals in the body fluids (e.g., blood, urine, etc.), the pertinent kinetic parameters can be established, and at times be used to back calculate to exposure levels. Furthermore, estimation can be made of the amount of substance metabolised and/or excreted in urine, feces, or expired air. Animal studies can be used to identify toxic metabolites and their concentrations in target organs. Measurements of changing enzyme activities can sometimes be used as biological monitors to estimate the magnitude of exposure to various chemicals.

In exposure assessment, biological monitoring is an important tool complementary to environmental monitoring. Ethical aspects of using biological monitoring in assessing human exposure have been discussed; the idea of using humans as walking sampling devices has been questioned. Also the drawing of blood samples has been criticised, since this procedure is not completely non-invasive and because the sample can also be used for other purposes. These viewpoints must be considered against the advantages of biological monitoring as compared to environmental monitoring. The latter does not account for skin absorption, bioaccumulation, use of personal protective devices, differences in physical activity, working habits, and personal hygiene, while the former does all this. Examples of common situations in which air monitoring is inadequate are contact of a chemical
with the skin, through which efficient absorption often occurs, and the failure of total air particle measurements to reflect the fact that the particle sizes of various dusts profoundly affect their deposition pattern in the airways and influence the extent of absorption.

4.1.3 DEVELOPMENT OF GENERAL STRATEGIES

Strategies for the use of a biological marker in research or disease control must begin with a clear understanding of the problem at hand and of the objective. Generic research questions and specific study hypotheses must be determined as much as possible; all biological hypotheses related to exposure/effect relationships should be delineated. The biological hypothesis or model is essential to the development of a strategy, since it guides the making of several decisions. The biological model depends heavily on knowledge of toxicokinetics.

Before starting a program, the following questions must be asked:

1. What is to be measured? What specific compounds are to be studied? Are there particular species of the toxicant (e.g., specific salts or valence forms of metals or reactive metabolites) that are critical to the model?

2. What level of quality of the data is needed? Does the research question call for precise measurements or qualitative ones (as, for example, in studies of urinary mutagens among oncology nurses)?

3. Who is to be studied? Where are the exposed populations appropriate for study? What ethical and legal questions exist? Are there subgroups potentially at increased risk? Can occupational groups or patients be studied as reliable surrogates for the general population?

4. When are samples to be taken? According to the biological model, at what period relative to the exposure should samples be taken? How long a duration of exposure should these samples represent? How often must sampling be repeated?

5. Which analytical techniques should be used on collected samples? What are the advantages and limitations of alternative methods?

6. At what locations should measurements be made? Given the above considerations and the general criteria for markers, which biological material should be sampled?

Answers to these questions guide criteria for the evaluation of potential markers. These criteria must be explicit. If each proposed marker is carefully evaluated, much waste and conflict can be avoided.

Two examples of planning strategy for the purpose of biological monitoring are described below:

1. Cadmium (Cd). Cd is a cumulative toxic chemical with a biological half-life in humans exceeding ten years. Liver and kidneys are the two main sites of deposition. Following long-term exposure to Cd, the kidneys are
the critical target organs. Renal tubular dysfunction leading to excessive urinary excretion of low molecular weight proteins occurs when the concentration of Cd in the renal cortex has exceeded a critical level. The concentration of Cd in liver and in kidney can be measured in vivo by neutron activation analysis. Before the occurrence of renal dysfunction, the concentration of Cd in urine is a reliable reflection of the amount of Cd stored in kidneys. The concentration of metallothionine in urine has the same biological significance as urinary Cd. Cd concentrations in blood represents both recent exposure and the amount stored in the organism. Under moderate exposure conditions (e.g., in occupational settings), blood-Cd is mainly a reflection of recent months of exposure. But in workers removed from exposure for several years, the body burden of Cd will be the main contributor to blood-Cd.

(2) Benzene. Biological monitoring of exposure to benzene was reviewed by Berlin (1984). The primary monitoring methods for benzene measure the urinary metabolite, phenol (free or conjugated), as well as benzene itself in exhaled air and blood. A test for the presence of phenol in urine is not sufficiently sensitive to detect low levels of benzene exposure, and is a non-specific indicator of exposure to toxic substances. Benzene measured in expired air reflects exposure only at the time of sampling; the benzene content of breath the morning after exposure best reflects the integrated internal dose of the preceding day. Determination of benzene in blood is specific and, thus, is the method of choice for low levels of exposure to the compound. At equilibrium in the body, bone marrow, the target organ for benzene toxicity, retains 20 times as much benzene as blood. Benzene is a known human leukemogen, produces a decrease in circulating blood counts and has been shown to cause chromosomal aberration. All these parameters are part of toxicokinetic measurements for benzene.

4.1.4 QUALITY ASSURANCE

Quality assurance (QA) should be an integral part of biological monitoring in all experimental studies. Quality assurance refers to steps taken to ensure that laboratory analyses are carried out in accordance with standard operating procedures and that the data quality objective is in keeping with the overall aim of the program. Part of QA is internal quality control, e.g., the procedures within a laboratory to assess analytical results to decide their reliability and acceptance by the scientific community. Without continuous internal quality control, a responsible scientist should release no laboratory findings.

External quality control is now used for objective evaluation of laboratory performance by governmental or other organisations. Although external quality control is presently a routine practice in clinical chemistry, quality control for biological monitoring in experimental laboratories is in its infancy.
4.1.5 CONCLUSIONS

(1) Knowledge and use of toxicokinetics is important in quantifying biological exposure for most substances; the four W's are necessary determinants: why, where, when, and what to quantify.

(2) Certain information is a prerequisite to the initiation of a biological monitoring program, such as the extent of environmental exposure of a substance under investigation.

(3) The objective of biological monitoring may vary in different studies, usually it has a preventive purpose.

(4) Quality control including appropriate analytical and statistical methods must be an integral part of a biological monitoring program.

(5) Toxicokinetics are a key factor in establishing exposure assessment approaches and providing the basis for biological monitoring strategies.

4.1.6 RECOMMENDATIONS

(1) For many chemicals, toxicokinetic data (absorption, distribution, excretion and target organ accumulation) are not available. A list of priority chemicals should be prepared, and toxicokinetic data should be developed for compounds on the list.

(2) Further knowledge is needed about reactive intermediates, their reactions in the body, and their use in biological monitoring of exposure.

(3) Quality assurance has not yet been well established for all aspects of biological monitoring. The interlaboratory quality assurance program should be expanded, and all biological marker research centers should be integrated into a comprehensive QA network.

4.2 DETERMINATION OF INTERNAL DOSE

4.2.1 ANALYSIS FOR ELEMENTS

Internal exposure to metallic compounds is often estimated by determining the level of the metal or element in biological materials (for examples, see Table 4.1). However, such methods do not differentiate among different chemical forms (e.g., inorganic and organic mercury, different valence forms of chromium), which can greatly influence toxicity. Accordingly, the form in which the metal is present in the biological material should be determined where possible.

X-ray fluorescence and neutron activation analysis techniques can determine metals in human tissues in vivo, and they provide new opportunities to assess long-term internal exposure (i.e., body burden) to certain metals (e.g., Cd in kidneys and liver). The development, calibration and validation of such methods should be encouraged.
Methods for Assessing Exposure of Human and Non-Human Biota

### Table 4.1. Illustrations of biological materials and analytical methods to monitor exposure to inorganic elements

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Recent exposure</th>
<th>Long-term exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>Plasma (AAS)</td>
<td>Bone (AAS)</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Urine (AAS)</td>
<td>Kidney cortex (AAS, ASV)</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Blood (AAS, ASV)</td>
<td>Liver (AAS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urine (AAS)</td>
</tr>
<tr>
<td>Chromium</td>
<td>Urine (AAS)</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td></td>
<td>Plasma (AAS)</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>Serum/Plasma (AAS)</td>
<td>Teeth (AAS; SEM-X-Ray)</td>
</tr>
<tr>
<td>Lead (inorg)</td>
<td>Blood (AAS, ASV)</td>
<td>Bone (XRF)</td>
</tr>
<tr>
<td>Mercury (inorg)</td>
<td>Blood (AAS)</td>
<td>Hair (AAS)</td>
</tr>
<tr>
<td>Nickel</td>
<td>Urine (AAS)</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>Urine (AAS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma (AAS)</td>
<td></td>
</tr>
</tbody>
</table>

AAS = Atomic Absorption Spectrometry  
ASV = Anodic Stripping Voltammetry  
NA = Neutron Activation Analysis  
SEM = Scanning Electron Microscopy  
XRF = X-Ray Fluorescence

4.2.2 ANALYSIS OF BIOLOGICAL MATERIALS FOR PARENT CHEMICALS AND THEIR METABOLITES

Many chemicals require metabolism to produce their toxic effects. For such chemicals, it is more relevant to measure internal exposure to metabolite(s), rather than to the parent chemical. Another approach is to estimate internal exposure by measuring the amount of metabolite bound to a macromolecular receptor (e.g., DNA adducts of some carcinogens). However, many active metabolites have such short half-lives (sometimes in the order of milliseconds), that it is not feasible to determine them in biological materials.

In cases where the parent chemical, and not a metabolite, is responsible for the biological effect of concern, it is preferable to estimate internal exposure to the parent chemical or to measure the amount of chemical bound to macromolecular receptor(s) (e.g., alkylating agents bound to DNA). However, when the parent chemical is unstable or is metabolised very rapidly, it may be more practicable to estimate internal exposure by analysing biological material for the metabolite and subsequently calculating exposure to the parent chemical from the results. This approach has been widely used to obtain an indication of recent exposure to certain volatile
BIOLOGICAL MONITORING OF EXPOSURE TO CHEMICALS

chemicals of concern in environmental and occupational health (e.g., nicotine, formaldehyde, and styrene).

Examples of biological materials and analytical methods that can be used for monitoring chemicals determined as such or as metabolites are given in Tables 4.2 and 4.3, respectively. The examples given in these tables are not necessarily the chemicals of major interest, but were chosen from material available at the Workshop and the knowledge of the participants in the subgroup.

4.2.3 RECOMMENDATIONS

Research aimed at improving methods to determine the chemical form in which metals are present in biological materials, especially those of interest for biological monitoring, should be encouraged.

Table 4.2. Illustrations of biological materials and analytical methods that can be used for monitoring exposure to chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Recent exposure</th>
<th>Long-term exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylonitrile</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Alkyl mercury cpds</td>
<td>Whole blood (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Benzene</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Chlordane</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>DDT, DDE</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>HCB</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>HCH-isomers</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Methylene chloride</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Blood/Plasma</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>PBBs</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>PCBs</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>PCDDs</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>PCDFs</td>
<td>Blood (GC, GC/MS)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>PCP</td>
<td>Serum/Plasma</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Styrene</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>Urine (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Tetrachloroethane</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Tetrachloroethene</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Toluene</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Trichloroethane</td>
<td>Blood (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Vinylidene chloride</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
<tr>
<td>Xylenes</td>
<td>Exhaled air (GC)</td>
<td>Adipose tissue (GC)</td>
</tr>
</tbody>
</table>
### Table 4.3. Illustrations of biological materials and analytical methods that can be used for monitoring exposure to chemicals by investigating their metabolites

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Recent exposure</th>
<th>Long-term exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>Dieldrin in blood (GC)</td>
<td>Dieldrin in adipose tissue or breast milk (GC)</td>
</tr>
<tr>
<td>Arsenic, Inorganic</td>
<td>Monomethylarsenic acid &amp; dimethylarsenic acid in urine (AAS)</td>
<td></td>
</tr>
<tr>
<td>(water-soluble)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>Phenol in urine (GC)</td>
<td></td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>N-Methylformamide in urine (GC)</td>
<td></td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>Hydroxyethoxyacetoacetic acid in urine (GC)</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>Cotinine in urine or saliva (RIA, GC)</td>
<td></td>
</tr>
<tr>
<td>Styrene</td>
<td>Mandelic acid in urine (GC)</td>
<td></td>
</tr>
<tr>
<td>Toluene</td>
<td>Hippuric acid in urine</td>
<td></td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>TCA in blood or urine</td>
<td></td>
</tr>
</tbody>
</table>

### 4.3 BIOLOGICAL EXPOSURE MARKERS

Biological markers of exposure are indicators of internal dose arising through absorption, distribution, and biotransformation. Markers include alterations in enzyme activity, covalent adducts with cellular macromolecules, and disturbances in cell structure, function, and/or dynamics. It is also desirable for biological markers of exposure to be early indicators of chronic toxicity, carcinogenicity, or mutagenicity.

Preferably, biological markers should be specific and quantal, although many of the procedures currently in use are non-specific and dose-proportional. They should be related to each target organ and its function; for humans, however, they are usually obtained from samples (e.g., urine, blood, feces, cerumen, or saliva) that are easily accessible by non-invasive techniques. By contrast, for non-human targets, access to target organs may be more extensive.

Changes in morphology, growth, development, lifespan, or in the inherent capacity of the target organism to cope with additional stresses has provided hitherto the basis for recognising adverse effects of environmental chemicals. However, ever since the concept of the “biochemical lesion” was proposed by Peters (1969), there has been a continuing search for those subtle changes in cellular metabolism and related functional activities which could distinguish between non-adverse effects and eventually irreversible adverse effects.
Ideally, for a biochemical lesion to be predictive and, therefore, to be used as a biological marker, it should detect early metabolic alterations which, if allowed to proceed unchecked, would produce pathological alterations and physiological dysfunctions. The concept of "biochemical lesion" implies that the cellular or molecular mechanism for time-dependent evolution of a non-injurious effect into an adverse one is understood. Indeed, it was the discovery of the competitive inhibition of a vital enzyme that logically led to the development of the antidote BAL.* However, the situation presented by the chronic toxicity of environmental chemicals of current global concern embodies a complex set of phenomena, and as yet few leads are available to unfold the underlying molecular mechanisms. Thus, for renal toxicity associated with chronic cadmium exposure, the pathophysiological changes in renal tubular function are manifest as increased output of beta-2-microglobulin or retinol carrier/binding protein in the urine. Although there is a presumably dose-dependent cause/effect relationship for this phenomenon, the mechanism of Cd toxicity is still not fully understood. Nevertheless, the measurement of urinary output of specified polypeptides is practical for a biological marker for Cd exposure and toxicity.

It is worthwhile to ask some basic questions about the specificity of biological markers. Does urinary output of excessive amounts of beta-2-microglobulin represent a specific biological marker of Cd toxicity? Obviously not, because much of the same phenomenon is encountered with a number of structurally unrelated inorganic and organic renal toxicants. However, in certain situations where Cd has been identified, the pattern and profile of urinary beta-2-microglobulin can be refined and adapted as a biological marker.

Generally the same conclusion can be reached with regard to the specificity of other biological markers which have been extensively used in the clinical diagnosis of diseases. The achievements of automated instrumentation in quantifying changes in the levels of blood cells and serum enzymes are indeed very impressive. However, in practice, these tests have proved to be of much greater value in confirming the diagnosis of diseases rather than in their early detection. Here also, changes in the activities of marker enzymes or changes in the profile of isoenzymes can be considered at best as group specific or target specific markers. One of the few known specific biological markers is the formation of carboxyhaemoglobin (COHb) by carbon monoxide (CO). COHb is both a marker of integrated exposure during the previous 8 to 12 hours and a marker of an effect directly on the pathway of the known mechanism of CO toxicity.

Induction of hepatic mixed function oxygenases by environmental chemicals can be monitored by estimating the half-life of a biotransformed marker chemical administered orally. This technique, however, has not found wide

* BAL = British Anti-Lewisite.
METHODS FOR ASSESSING EXPOSURE OF HUMAN AND NON-HUMAN BIOTA

application in exposure related toxicity studies in humans. Currently, no method exists to measure the activity of cytochrome P-450 species can be measured by non-invasive techniques except the indirect method of calculating ratios of the marker compound to its main urinary metabolite.

The correlation observed between blood levels of lead and RBC levels of aminolevulinate dehydratase or blood levels of cadmium and urinary excretion of beta-2-microglobulin can be attributed to a relatively improved understanding of the mechanism of toxicity of these two metals. For mercury, chromium, nickel, or manganese, the molecular mechanisms of toxicity remain to be elucidated; and viable biological markers do not exist.

Neurotoxicity is a serious endpoint of the chronic effects of lead, mercury, and manganese. Behavioural effects are also associated with these metals. Measurements of electromyographic responses or nerve conduction velocities are useful only when adverse effects have already occurred. It is unclear whether the quantitative changes revealed by such measurements can be used to detect a biochemical lesion preceding neuropathic effects. Plasma levels of the diverse groups of neurotransmitters or their metabolites in urine are potential markers for neurotoxicity but have not been validated by epidemiological studies.

In addition to the use of markers to assess exposure versus early response in the nervous system, liver, and kidney, recent developments in research on the lung point to opportunities for developing markers that arise from viable models of disease mechanisms. In particular, considerable evidence has recently been assembled about the role of oxidants, proteases, and various defenses against microscopic lung injury leading to emphysema or fibrosis. It is hypothesised that imbalances between proteases and anti-proteases and between oxidants and anti-oxidants lead to biochemical injury of collagen and elastin, the major chemical proteins in pulmonary connective tissue. Irritants such as ozone or the diverse components of cigarette smoke injure this tissue both directly and indirectly through inactivation of anti-proteases. Ethylene in exhaled air as a measure of lipid peroxidation, oxidant effects on glutathione and cell membranes, cleavage of anti-proteases by oxidants at the methionine reactive site or measurement of circulating protease+anti-protease complexes, and detection of elastin peptide fragments in serum or hydroxyproline in urine—all suggest themselves as potentially valuable markers for oxidant exposure (Gann, this volume).

4.3.1 BIOLOGICAL MARKERS AS INDICATORS OF EXPOSURE TO CARCINOGENS AND MUTAGENS

Recently, new procedures have been developed that could identify individuals or populations exposed to carcinogens or mutagens. The majority of these procedures are designed to detect agents that interact with DNA directly or indirectly. This topic has been reviewed recently by Garner (1985), and
has been the theme for an international conference (Berlin, 1984). The methods can be divided into specific and non-specific. Table 4.4 lists the methods currently under study and their advantages and disadvantages. Ideally, assay procedures should be predictors of carcinogenicity or mutagenicity, such that the results of the determination could lead to reduction in exposures as a means of disease prevention. No current assay is capable of this directly, although this might be possible some day. Assay procedures should be sensitive, quantitative, inexpensive, rapid, reproducible, and non-invasive. Presently, all listed methods indicate internal exposure, but none is a direct indicator of the final biological endpoint, namely cancer or mutation. It is unclear whether covalent adducts, chromosome aberrations, and other measures are adverse to the organism.

**Table 4.4. Biological markers used to measure exposure in non-human targets**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chemical</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible lesions</td>
<td>Airborne pollutants, e.g., O₃, NOₓ, SOₓ, F, etc.</td>
<td>Some higher plants are sensitive. Difficult to establish dose/effect relationship</td>
</tr>
<tr>
<td></td>
<td>Pollutant aromatics</td>
<td>Tumours in fish, semi-qualitative relationship</td>
</tr>
<tr>
<td></td>
<td>Dioxins</td>
<td>Birds with crossed beaks etc. (semi-qualitative at best)</td>
</tr>
<tr>
<td>Genetic markers</td>
<td>Air pollution (smoke)</td>
<td>Melanism in moths, adaptation to darker areas</td>
</tr>
<tr>
<td></td>
<td>Mutagenic compounds</td>
<td><em>Drosophila</em> mosaic test, sister-chromatid exchange, etc.</td>
</tr>
<tr>
<td>Immunological</td>
<td>Microbiological insecticides</td>
<td>Immunological changes in insects</td>
</tr>
<tr>
<td>Cytological</td>
<td>Pesticides, active metabolites</td>
<td>Mitochondrial swelling, disaggregation of polysomes, aggregation of chromatin in insects</td>
</tr>
<tr>
<td>Acetylcholinesterase inhibition</td>
<td>Organophosphates</td>
<td>Readily related to exposure. Can be used over a wide range of taxa</td>
</tr>
<tr>
<td></td>
<td>Carbamates</td>
<td></td>
</tr>
<tr>
<td>Induction of hepatic mixed function oxidase</td>
<td>Organochlorines</td>
<td>Rather non-specific, Levels of induction low in lower forms (in insects)</td>
</tr>
<tr>
<td></td>
<td>Polynuclear aromatics</td>
<td></td>
</tr>
</tbody>
</table>
or of no health significance. Studies utilising animal models should enable
the predictive value of the different methods to be tested.

4.3.2 BLOOD ANALYSIS FOR BIOLOGICAL EFFECTS OF
MUTAGENS AND CARCINOGENS

Blood samples, although requiring semi-skilled personnel and sterile
equipment to obtain, appear to be one of the easiest of the body fluids to
recover for analysis.

4.3.2.1 Non-specific Biological Exposure Markers

*Chromosome Aberrations in Peripheral Lymphocytes* Microscopic visualisation
of metaphase chromosomes is performed relatively simply and, although
requiring trained personnel for scoring, can be carried out in relatively
primitive laboratory conditions. Microscope slides can also be easily
interchanged, so that aberration frequencies can be confirmed independently.
Increased levels of chromosomal aberrations have been detected in a variety
of persons (Vainio and Sorsa, this volume) exposed to occupational
chemicals, ionising radiation, cigarette smoke, and alcohol. Since many
human tumours have abnormal chromosomes, it might be argued that
detection of aberrations in peripheral lymphocytes indicates precancerous
lesions. There is no evidence to date to confirm the view that chromosomal
abnormalities are the cause of any subsequent tumour.

Considerable difficulties are associated with the use of chromosomal
aberration monitoring to detect exposure to environmental toxicants. Low
level exposure may induce an increased frequency of aberrations; but this
modification is difficult to detect statistically, probably because the noise in
the system is larger than the number of compound-induced aberrations.
Furthermore, monitoring chromosomes in circulating lymphocytes is not an
indication of target organ dose, or of toxic effects, since the damaged cells
will die. Also, chromosomal damage cannot be attributed to any particular
environmental pollutant, since the injury is non-specific. Thus, aberration
monitoring in occupational settings is of questionable value for agents
demonstrated to be clastogenic *in vitro*, regardless of whether scoring could
be automated and despite the apparent longevity of lymphocytes with
aberrant chromosomes.

*Sister Chromatid Exchanges (SCE) in Peripheral Lymphocytes* Exchanges
arise through recombinant events between chromatids. These recombined
chromatids are scored by a variety of techniques. The biological significance
of the process is unclear. Agents that cause DNA double strand breaks
such as X-rays and bleomycin induce chromosomal aberrations but not SCE.
Scoring exchanges is somewhat tedious, and can be undertaken by staff less
skilled than required to score aberrations. Chromosomal exchanges can be
detected at lower concentrations than can aberrations.
Frequency of chromosomal exchanges varies among individuals and within the same individual depending upon sampling time. Increases in exchange frequency tend to be small, so that large numbers of chromosomes must be scored to achieve adequate statistical power. Even where individuals are exposed to potent alkylating agents, such as those used in chemotherapy, or to cigarette smoke, only a small elevation in exchange frequency can be observed. Automation of scoring might be achieved by image analysis, thus increasing sample throughput. The specificity of SCE is as limited as for chromosomal aberrations.

**Micronuclei** Micronuclei arise from failure to segregate chromosomes normally during mitosis. A small piece of a chromosome might break off as a result of damage, and become encapsulated in its own nuclear membrane. A strong correlation has been reported between micronuclei induction and chromosome aberrations (Van Sittert and DeJong, 1985), and the induction of micronuclei in buccal mucosa cells has been related to betel nut chewing (Stitch and Rosin, 1984).

"Unscheduled" DNA Synthesis in Peripheral Lymphocytes DNA damage by either chemicals or ionising radiation results in repair by the cell. A variety of enzymes capable of DNA repair have been identified; undoubtedly others will be discovered in the future. Differences in repair capacity have been found among individuals, organs, and cells and cell types. These differences are likely to incorporate much variability in studies of DNA repair. Nevertheless, attempts have been made to demonstrate the presence of "unscheduled" DNA synthesis in occupationally exposed groups as well as in patients undergoing anti-cancer treatment. Repair synthesis can be assayed by incorporation of \([^3]H\)thymidine, alkaline elution, and by alkaline sucrose centrifugation.

An increase in DNA repair synthesis probably results from acute exposure to a DNA-damaging agent and, therefore, does not reflect chronic exposure. DNA repair synthesis is likely to be a non-specific response. An additional complication is that the background level of DNA repair appears to vary daily in the same individual. This is another assay in which the signal-to-noise ratio is likely to be too low to be useful in measuring carcinogen exposure.

**Mutation Screening in Blood Cells** With the ability to culture human lymphocytes *in vitro*, it is now possible to examine cells *in vivo* for induced mutations. The most widely used procedure examines isolated cells for resistance to agents such as 6-thioguanine. Resistance arises through the inactivation of an X-linked gene through point mutation, deletion, or frame-shift mutation. Methods using this technique are in the experimental stage; problems encountered include what appears to be a high spontaneous mutation rate. Anticipated problems include failure of activated metabolites to reach the target DNA, faithful repair of adducts eliminating the damage, and possible insensitivity of the target T-cell sub-population. In addition to
the study of mutations in lymphocytes, some papers have reported the
induction of haemoglobin variants in red blood cells. The use of restriction
fragment polymorphism might also be a useful method to demonstrate DNA
alterations arising through carcinogen damage.

4.3.2.2 Specific Methods of Macromolecular Adduct Determination

Many organic chemical carcinogens and mutagens are reactive species
which chemically attack cellular macromolecules. Indeed, the electrophilic
hypothesis proposed originally by the Millers is that this property is
fundamental for the majority of chemical carcinogens. Relying on radio-
labelled tracers, animal studies have demonstrated adducts bound to all
cellular nucleophiles in a variety of organs and cell types. Relatively few
studies have been reported on adduct levels in blood since this is not usually
the target of interest. Recent reports suggest that adduct formation in blood
may be more common than expected (Pereira and Chang, 1982).

Haemoglobin Alkylation Administration of a variety of radio-labelled
carcinogens to animals results in the adduction of haemoglobin (Pereira and
Chang, 1981). By recovering the haemoglobin, digesting it and performing
gas chromatography/mass spectrometry, it has been possible to demonstrate
amino-acid conjugates with methyl-alkylating agents, ethylene oxide and
ethylene (Ehrenberg and Osterman-Golkaa, 1980). Haemoglobin adducts
have also been detected by mass spectrometry in animals dosed with
4-aminobiphenyl (Skipper et al., 1986). Activation of the chemical appears
to be catalysed by red blood cell enzymes.

Dose/response studies have related hydroxy-ethylation of haemoglobin
with that in liver DNA after ethylene oxide exposure. Studies in humans
have indicated that there is a basal level of hydroxy-ethylation of
haemoglobin; the source of this is unknown, but clearly complicates attempts
to relate occupational exposure to ethylene oxide or to ethylene with the
concentration of haemoglobin adducts.

Although there appear to be advantages to the use of haemoglobin adducts
as a monitor of internal exposure (e.g., the long lifetime of the red blood
cell), the methods of analysis are extremely laborious and require highly
sophisticated equipment. Consequently, the method is unlikely to lend itself
to population monitoring. Also, limited experience with carcinogens such
as benzo(a)pyrene and aflatoxin B1, show only low levels of haemoglobin
adducts in animals. The ability of the red blood cell to activate other
carcinogens requires rigorous examination. Immunological methods to
detect haemoglobin adducts might also be used.

Other Protein Adducts The ability of carcinogens or their metabolites to
react with serum proteins might also be used to monitor for carcinogen
exposure. Reactions with serum albumin could be an appropriate monitor,
particularly as this protein is synthesised in the liver, the site of many biotransformation reactions. Limited evidence indicates that albumin can be covalently bound by diverse carcinogens including aflatoxin B1, acetylamino-fluorene, and dimethylnitrosamine. The problem with the measurement of albumin reaction product is that protein turnover is relatively rapid. Nevertheless, in chronic exposure conditions, a steady-state level should be reached between protein turnover and binding levels. Recently, a constant ratio has been found between serum albumin and liver DNA binding of aflatoxin B1 in rats both under acute and chronic exposure conditions. Protein binding can be assayed either by physico-chemical or immunological methods. Blood-protein binding studies appear to be a fruitful avenue of research.

Reactions with DNA

The biologically important target molecules for carcinogens and mutagens in DNA+protein adducts are measured not because they are essential to the cancer process, but because a relationship could exist between levels of protein and DNA binding. Until recently, no techniques were available to estimate levels of DNA adducts without the use of radio-labelled tracers. Performance of this type of study in humans cannot be justified except for diagnostic purposes. Three types of methods have recently been described which could enable DNA adducts to be studied in humans:

1. $^{32}$P Post-labelling Methods. When a carcinogen reacts with DNA, it usually forms a stable covalent adduct that can be isolated. Many DNA adducts with carcinogens have been structurally characterised \textit{in vitro} by the reaction of chemically synthesised reactive metabolites with DNA, and subsequent enzyme digestion and chromatography. Randerath and his colleagues (Gupta \textit{et al.}, 1982) have published a method that relies on the base-bisphosphate+carcinogen adduct having chromatographic properties different from unreacted base-bisphosphates on TLC. The technique involves DNA extraction, enzymatic digestion to mononucleotide monophosphates and subsequent phosphorylation using [32P]ATP and polynucleotide kinase. The resulting bisphosphates are chromatographed by 4-dimensional chromatography. Adducted bisphosphates thus are separated from unreacted bisphosphates, and can be revealed by subsequent autoradiography of the plates. The method has been successfully applied to DNA from target organs of animals treated with aromatic amines and polynuclear aromatic hydrocarbons (PAH). The technique works well for non-polar adducts that are well separated from unreacted bases. Problems have been encountered with DNA adducts of alkylating agents and aflatoxin B1. PAH-adducts have been demonstrated in placental DNA of cigarette-smoking mothers.

This method suffers from the disadvantage that marker compounds are required. Digestion of DNA from an individual exposed to a variety of carcinogens could lead to a number of spots on the resulting chromatogram.
which cannot be identified. Even with marker compounds, it is possible that a completely unrelated adduct might have the same \( R_f \) value. In the long run, the method may have limited usefulness.

(2) Antibody Methods. Antibodies are likely to provide one of the most useful tools for measuring carcinogen+DNA adducts. Antibodies are specific to the antigen against which they were raised, can have high affinity, and can be used for immuno-concentration. Both radioimmunoassay and enzyme linked immunosorbent assay techniques have practical application. The method is dependent on

(a) chemically characterising the DNA adduct of interest,
(b) being able to synthesise sufficiently immunogenic material, and
(c) developing a suitable assay procedure.

Useful antibody preparations can be either mono- or polyclonal. Each has its advantages and disadvantages for screening. Mono- and polyclonal antibodies have been generated against carcinogen-reacted DNA and protein conjugates with carcinogen + base adducts (Poirier, 1981). Antibodies have been used to measure methylguanamine in esophageal tissue from patients in China (Umbehaven et al., 1985). Human lung tissue removed at surgery has been examined for the presence of benzo(a)pyrene adducts: some individuals analysed had detectable adduct levels. Rabbit polyclonal antibodies have been raised against cis-platinum adducts, and have been used to measure adduct levels in lymphocytes from patients undergoing treatment. Methods to improve sensitivity include the use of immunoaffinity columns to concentrate adducts, which have been used successfully to measure benzo(a)pyrene DNA adduct levels in mouse skin (Santella et al., 1985). Although antibodies appear to offer the ideal method for studying carcinogen/DNA reactions at the cellular level, there are certain practical limitations. If crude mixtures are tested, there is always the possibility of non-specific antibody inhibition. It is usual to carry out preliminary clean-up procedures and preferably have some confirmatory technique available such as mass spectrometry, or another antibody recognising some other epitope in the material to be analysed.

Target DNA molecules capable of being studied include some found in lymphocytes and granulocytes. The combination of immunological methods with population studies can provide a measure of internal carcinogen exposure that might be eventually correlated with the production of cancer. However, it is not yet possible to conclude that because \( X \) number of adducts have been detected in DNA the risk of developing cancer is \( Y \).

(3) Physico-chemical Methods. Some carcinogen+DNA adducts fluoresce strongly, and have characteristic excitation and emission spectra. This property has been exploited to study benzo(a)pyrene+DNA adducts in alveolar macrophages lavaged from human lungs. The method may have application to other carcinogen moieties which also are fluorescent, such as aflatoxin B\(_1\).
4.4.2.3 Urine and Faecal Analysis for Biological Effects of Mutagens and Carcinogens

The result of the biotransformation of foreign compounds is their excretion primarily in the urine and faeces. In addition, covalently bound adducts of cellular macromolecules are likely to be degraded eventually and excreted. A number of specific and non-specific methods have been utilised to study these events in the hope that they will give some measure of carcinogen exposure. Current methods using urine and faeces to provide quantitative, as well as qualitative, information are limited by their failure to take toxicokinetics into account. Methods used to study urine and faeces include bacterial mutagenicity (National Toxicology Program, 1984) and adduct analyses using physico-chemical and immunological methods.

**Urine and Faecal Bacterial Mutagenicity** Animals dosed with various carcinogens have been shown to have mutagenic urine (Dolama et al., 1981). For dimethylaminoazobenzene, it is possible to detect mutagenicity in the urine of dosed animals, despite the difficulty in showing that the compound is a bacterial mutagen *in vitro*. Such a situation is likely to be the exception rather than the rule, as urine and faecal metabolites are usually detoxification products, and would be expected to be less mutagenic than the activated species. Urinary and faecal mutagens in humans can also arise through the diet. They also may be endogenously synthesised; and they can result from occupational or environmental exposure (Dolama et al., 1981). Methods and results of performing mutagenicity studies have been well described in the literature.

Except for heavy occupational exposure, the association between the presence of bacterial mutagens and a particular environmental factor is unlikely to be demonstrated. In the commonly used *Salmonella* tester strains, aromatic nitro- or amino-compounds could be easily over-represented, because of potent bacterial nitro-reductases. Furthermore, the results are usually only indicative of exposures over the previous 24 to 48 hours. Even in studies using well-controlled conditions, the results obtained have not been readily interpretable. The non-specific nature of bacterial mutagenicity tests is a disadvantage in determining exposure to a particular compound. Where the assay procedure could prove useful in the absence of other methods is in monitoring people under controlled conditions such as volunteers eating a particular diet or exposed to a specified environment. Novel genotoxicants might be detected using these procedures which could point to previously unsuspected exposure or to endogenous mutagen synthesis, e.g., faecapentane and endogenously nitrosated compounds.

**Adduct Analysis** The methods described for blood can also be applied to urine. Immunological methods require an antibody to be highly specific for the adduct under investigation, such adducts arising from either repair or degradation of reacted macromolecules. Assays can give misleading results,
because of the varying antibody affinities for metabolites and related adducts. This consequence can be circumvented by prior column chromatography to isolate the molecule of interest. Determination of whole body exposure to a carcinogen might be achieved by immuno-concentration of urine collected over a period such as a week giving an indication of chronic exposure. Urine and faecal analysis as a monitor of carcinogen or mutagen exposure could also give useful qualitative information.

Specific methods to detect well-characterised molecules by antibodies or physico-chemical techniques are preferred to non-specific procedures such as bacterial mutation tests.

4.3.3 BIOLOGICAL MARKERS AS INDICATORS OF EXPOSURE IN NON-HUMAN TARGETS

A variety of biological markers has been used in non-human targets; these were summarised in Table 4.4. In animals, two widely used systems are the induction of hepatic mixed function oxidases and the inhibition of acetylcholinesterase (AChE). The degree of biological variation of these enzymes is generally higher than for the determination of chemical residue levels in biological tissue; also, factors such as disease, nutritional status, and mineral deficiency may mask changes.

4.3.3.1 In Animals

The hepatic mixed function oxidase systems are known to be induced by a wide variety of compounds (Hart et al., 1963). From an environmental standpoint, the two most important classes of compounds are the organochlorines and the polynuclear aromatics. While induction of these systems indicates the presence of contamination, it does not specify the cause. Some limited degree of specificity may be achieved by using alternative substrates. The degree of induction varies among orders (Walker, 1980) but is generally too low in lower forms, e.g., insects, to be a useful technique in these organisms. The methodology is well defined for mammals, birds, and fish.

AChE inhibition specific to organophosphates and carbamates is much more readily measured than the chemical residues of the compounds causing the inhibition. It is possible to differentiate between the two classes of compounds by the degree of competitive binding (Martin et al., 1981).

4.3.3.2 In Terrestrial Plants

In higher plants, numerous biological responses can be used as indicators of exposure. Thus air pollutants may induce a variety of morphological and anatomical alterations in leaves, flowers, and fruits, including discoloration of various hues and patterns and malformations. Mineral deficiencies, insect
damage, and viral infections may also produce similar symptoms. However, quantitative exposure/effect relationships seldom exist. Nevertheless, the specificity of lesions caused by pollutants such as SO₂, O₃, F, and NH₃, and the availability of plant species or strains that are particularly sensitive to these agents have allowed the use of plant systems for environmental surveillance. Examples are tobacco (Bell-3) for O₃ monitoring and gladiolas for fluorine.

Growth reduction in higher plants can be used to detect the presence of air pollutants but the effects are not specific. The presence or absence of sensitive plants, such as lichen for SO₂, has been the basis of some pollution maps.

When considering both terrestrial and aquatic plants, the content and concentrations of a chemical among plant parts are important: e.g., shoots vs roots, leaves vs reproductive organs. The source of the chemical (soil or atmosphere) can sometimes be indicated from the shoot/root distribution.

Exposure can be assessed in terms of average concentration for the whole organism or some part (e.g., shell vs soft tissue), or per unit of lipid or protein. For plants, concentration should be expressed on a dry-weight basis in view of the often widely fluctuating water content.

The ability of certain taxa to accumulate some chemicals and to integrate absorption over time may be exploited to monitor the environment, illustrations of which are mussels in the marine environment (Galloway et al., 1983) and mosses and grass seedlings in ambient air.

### 4.4 ADVANTAGES AND LIMITATIONS OF BIOLOGICAL MARKERS

Biological markers can be viewed as tools for research or disease control, offering two distinct advantages to environmental health workers. In research, biological markers can provide cost-effective individual data that minimise numerous sources of variation (which function as biological sources of error) that intervene between external exposure and target dose or early effect. These sources of variation, both intra- and inter-personal, include differences in pharmacokinetics, physical activity, and anatomical relationships. Thus, as shown in Figure 4.4, they permit the researcher to deal with independent and dependent variables whose probability links are tighter than, for example, those between external exposure and clinical disease. In disease control, markers promise to provide new opportunities for early prevention.

The advantages of biological markers in environmental health can best be illustrated by examining how they respond to major methodological problems currently faced by various disciplines.
4.4.1 EPIDEMIOLOGY

The most serious methodological problem in environmental epidemiology amounts to what might be called a "sensitivity crisis" (Gann et al., this volume). Stated differently: Are current epidemiological methods sufficiently sensitive to detect quantitatively associations between low-level chemical exposure and relatively small but widespread risks of chronic disease? The sample sizes required theoretically might be prohibitive. In this context, markers of exposure offer four advantages:

1. Selecting and defining populations for study: Biological markers permit epidemiologists to conduct population surveys that may be used to determine whether the magnitude of chemical exposures warrants concern and to define specific subpopulations in whom analytical studies might be fruitful. Focussing epidemiological studies on high-risk populations enhances their statistical power, i.e., detection of an exposure/disease association is possible with fewer subjects.

2. Reducing misclassification errors: The likelihood of error in assigning subjects to correct exposure level categories (misclassification error) is high, particularly when long-term low-level exposures are involved. This situation is due in part to uncertainties concerning those factors that influence the relationship of external exposure to internal target dose. Misclassification usually blurs the distinctions between exposure level categories, thus biasing a study towards the null hypothesis and making it less able to detect small but important associations. Even if a marker provides a poor reflection of long-term doses, it can be used to reduce misclassification by validating in part exposure models based on environmental measurements; for example, by validating the ranking of various jobs in a workplace by exposure intensity.

3. Integration of multiple routes and sources of exposure. Biological markers obviously provide an indication of total exposure to an individual when multiple sources or routes (such as food, air and water) are involved. This can obviate the need for environmental measurements of each potential source.

4. Delineation of mixture components and covarying exposures. Most studies in environmental epidemiology are observational in nature, based upon naturally-occurring exposures to populations. Exposure to complex mixtures (e.g., toxic waste) or multiple, covarying substances (e.g., urban air pollution) often present problems, because the detection of associations between specific individual pollutants and adverse health effects is desirable from both scientific and regulatory points of view. In theory, some biological markers can indicate which components are actually reaching and altering target sites. Markers can sometimes combine and separate diverse elements of exposure. For example, blood-Pb accounts for exposure via inhalation or
ingestion; measurement of Pb isotope ratios in blood can determine the fraction attributable to leaded gasoline sources.

4.4.2 ANIMAL TOXICOLOGY

Five ways can be defined in which biological markers might alleviate methodological problems in animal experiments:

(1) Increasing the feasibility of studying low-level exposures. Animal experiments must often use exposures higher than those likely to occur in the environment because of cost constraints in studying large numbers of animals. This problem is exacerbated when the experimental animal itself is rare and expensive, e.g., primates. These situations often require extrapolation of findings from higher to lower doses. By providing increasingly sensitive measurements of relationships between low-dose and early response, markers can permit the study of lower exposures with fewer animals.

(2) Exploring the relationship of acute to chronic insult: It is sometimes presumed that chronic environmentally-induced disease is the result of repetitive insult to the target tissues. Biological markers can assist the toxicologist to explore similarities between the acute and largely reversible response to a toxicant and the responses indicating chronic irreversible damage; for example, the relationship between acute haemodynamic changes following carbon monoxide exposure and progression of ischaemic heart disease. Once the links between acute and chronic insults are better understood, defining dangerous levels of exposure in humans will improve, and important opportunities for studies will surface. As an example of the latter, there are longitudinal studies in which the subject serves as his/her own control. Time-series analysis of the occurrence of reversible (but significant, in a predictive sense) micro-insults or molecular interactions in relation to variations in durations of exposure is a powerful aspect of study design being applied to acute toxicity such as asthmatic attacks. Such studies require participation of relatively few subjects.

(3) Interspecies extrapolation: Once the validity of a relationship between a dose marker and a biological response is established, inferences can be much improved for dose/response behaviour in various species (including humans), despite relatively incomplete data. With estimates of internal or target dose, large interspecies differences in toxicokinetics can be bypassed. Detection alone of an important dose or early response marker in humans or other free-living species can thus be related to exposures and outcomes in animal experiments, and can provide an improved means to assess risk to either human populations or ecosystems.

(4) Facilitating study of complex mixtures and multiple agents: Toxicologists now recognise the importance of designing experiments that involve exposure to complex mixtures or multiple agents rather than single chemicals. Protocols for such studies would be enhanced by markers capable of
delineating the significance of individual components or of clarifying the mechanisms of toxic chemical interaction. For example, studies of the effects of combustion products could be strengthened by biological markers indicating binding of specific agents to target sites on genetic material.

(5) Opportunities for less invasive animal experiments: The humane treatment of experimental animals is a matter of undisputed importance. Biological markers create more opportunities for non-invasive experiments, perhaps allowing significantly less animal deaths than when used traditionally. Researchers using these tools are also more apt to utilise the same animal in repeat experiments or separate protocols.

4.4.3 CLINICAL, PREVENTIVE, OCCUPATIONAL MEDICINE

The advantages of biological markers discussed below pertain to disease control activities:

(1) Improving detection of individuals at risk: Biological markers, once validated, can sometimes provide the clinician or preventive medicine specialist with a means for determining whether therapy or preventive action should be applied or whether searches for toxicants in the environment should be conducted. Some markers might eventually become accepted as clinical screening tests as have blood-Pb determinations in children.

(2) Determining significance of various routes of exposure: By measuring external routes of exposure (e.g., air) and biological markers simultaneously, occupational physicians can establish the relative importance of alternative routes of exposure. Corrective action can then be directed at the appropriate points. Some routes, such as dermal contact and absorption, can be difficult to measure in the environment.

(3) Assessing the adequacy of personal protective equipment, hygiene practices or environmental controls: Uptake of toxic chemicals often occurs in workers despite the use of protective clothing and respirators. Although measurements of substances within face-masks can sometimes be made, biological markers provide a relatively simple indication of whether protective devices have failed or whether individuals have failed to use them properly or not at all. Potentially dangerous deviations in personal hygiene practices can sometimes also be detected through dose markers. Finally, markers can provide an important complement to environmental sampling by ensuring the adequacy of environmental controls and of ad hoc exposure limits.

(4) Risk assessment: Biological markers—by generally strengthening the data base in toxicology, clinical research, and epidemiology and by specifically improving matters such as interspecies extrapolation—can make major contributions to qualitative and quantitative risk assessment. Quantitative risk assessment requires an understanding of the intrinsic hazard of a substance and extent of exposure of the human and non-human targets. While attention is often focussed on controversial aspects of hazard
assessment, it is often in the area of exposure assessment that rapid and cost-effective improvements can be made in the precise estimation of risk. Biological indicators of exposure are particularly valuable in exposure assessment, as they can provide accurate quantitation and integration of actual exposure. The development of biological markers that can link exposure and effect (e.g., a mutagenic DNA adduct) has the potential of allowing accurate estimates of risks at the lower end of the dose/response curve not attainable by standard epidemiological or animal testing procedures.

4.4.4 LIMITATIONS OF BIOLOGICAL MARKERS

The limitations of biological markers are summarised as follows.

(1) Ethical and legal constraints: To fulfil the ethical and legal aspects of a biological marker program, it is necessary prior to implementation to obtain approval and consent of the subjects, unions, administrations, authorities and whomever have a legitimate interest. Consequently, it is also necessary to clarify to interested parties the significance, risk, responsibilities, legalities, liabilities, possible loss of jobs, and possible adverse health effects. Assurance should be given regarding the privacy and secrecy of information collected. Lastly, the difficulties in interpretations which may limit and delay the feedback of the results, conclusions and recommendations need to be explained.

(2) Evaluation of biological markers: The expense and difficulty of evaluating a biological marker as a tool for research or disease control can limit the use of such tests.

(3) Persistence of a biological marker: Most known biological markers reflect short-term exposure or response rather than long-term phenomena, thus limiting their applicability in studies of chronic disease. Moreover, some short-term effects of highly reactive substances may not leave in the body a marker that can be maintained.

4.4.5 CRITERIA FOR BIOLOGICAL MARKERS

Biological marker research requires specific criteria to organise and establish programs for their assessment in the field of environmental health:

(1) Cost: Biological marker assessment for humans can be very costly because of the need for expensive equipment, highly qualified scientists, and relatively long follow-up periods. Some efforts should be directed at lesser cost methodologies, without sacrificing effectiveness.

(2) Invasiveness: Non-invasive procedures and techniques are generally preferred. Some degree of invasiveness or inconvenience to the subjects is inevitable, even though most subjects are asymptomatic or unexposed. Risk and discomfort should be minimised, lest they hinder the willingness of subjects to participate in the program. Retrospective study designs (e.g.,
case-control studies) can utilise markers from clinical or autopsy samples.

(3) Reproducibility: The markers should be repeatable in the same sample both within and between laboratories.

(4) Variation: A good marker should account for intrapersonal variation in measurements over time, considering factors in the host such as biological rhythm and diet. It should also account for interpersonal variation among individuals in the same examined group, in addition to variation introduced by the test agent itself. Failure to adequately understand major sources of variation can eliminate use of a marker or restrict its interpretation to group rather than individual comparisons.

(5) Temporal significance: It is important to know if a marker reflects recent or more distant exposures, peak or integrated exposure, and cumulative or non-cumulative effects. The temporal information conveyed by a marker must be consistent with the biological model or specific research question under consideration.

(6) Validation: Determining the validity of a potential marker requires calculation of its sensitivity, specificity, and predictive value in relation to quantities such as external exposure, target dose, and clinical disease. As opposed to calculations of simple correlation, these determinations involve (a) “gold standard” measurements not always available in humans, and (b) somewhat subjective decisions on transforming continuous data into categorical data. Furthermore, prospective epidemiological studies that require years for sufficient time to observe numbers of cases might not be feasible. Therefore, validation will often depend more extensively on the development of suitable laboratory animal models. As humans are usually exposed to much lower concentrations than those used in most laboratory animal studies, a valid marker must also be sensitive at low doses encountered typically by humans.

4.4.6 RELATIVE VALUE OF CHEMICAL ANALYSIS AND BIOLOGICAL RESPONSES FOR ECOLOGICAL MONITORING

Before any ecological monitoring program can be instituted, its purpose must be clearly stated. If the intent is to use wildlife as an early warning system for human safety, the program is likely to be different than if the objective is to protect wildlife. For the former, the likely routes of exposure and selection of appropriate indicator species are important; for the latter, it will depend on the wildlife resources that are to be protected. A monitoring program designed to protect the quality of a given ecosystem will be different from one designed to protect a specific species. A comparison between the two approaches is presented in Table 4.5.

The design of monitoring programs is also greatly influenced by the chemical(s) to be monitored. One extreme is to monitor a single chemical
### Table 4.5. Summary comparisons between chemical and biological monitoring for ecological systems

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Chemical Determinations</th>
<th>Biological Assays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproducibility of Assay</td>
<td>Generally more reproducible, and less seriously affected by factors, such as nutritional status, than are biological assays</td>
<td>Often comparable: for organophosphates, biological better; chemical better for organochlorines</td>
</tr>
<tr>
<td>Number/cost of Survey</td>
<td>Often comparable: for organophosphates, biological better; chemical better for organochlorines</td>
<td>As complexity increases, cost of analytical work increases and complete chemical analysis is not possible. However, there is no assurance that biological monitoring will be effective for all compounds</td>
</tr>
<tr>
<td>Complexity of Mixtures</td>
<td>As complexity increases, cost of analytical work increases and complete chemical analysis is not possible. However, there is no assurance that biological monitoring will be effective for all compounds</td>
<td>Chemical analysis may not differentiate between bioavailable and non-bioavailable chemical forms. Biological monitoring should respond to bioavailable chemicals only</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>Chemical analysis may not differentiate between bioavailable and non-bioavailable chemical forms. Biological monitoring should respond to bioavailable chemicals only</td>
<td>When the target is well defined, either chemical determining or biological monitoring could monitor risk. When system is complex, chemical monitoring alone is unlikely to monitor risk</td>
</tr>
</tbody>
</table>

The choice of indicator species and whether to use biological responses or chemical techniques depends on the basic reason for the monitoring program and on the chemicals of interest. A basic difficulty of any monitoring program designed to protect environmental quality is the large number of potential target species. This is a problem whether biological or chemical techniques are used. The second basic difficulty is the large number of chemicals that may be involved; one is rarely considering a single chemical, and the more usual case is a complex mixture whose composition varies greatly spatially and temporally.

Wide variations in species sensitivity to specific chemicals have been observed. For example, shrimp are eliminated by DDT at concentrations orders of magnitude below those which affect oysters. Some species of raptoral and fish-eating birds were eliminated from eastern North America by DDE-induced eggshell thinning while other avian species were unaffected. These variations in species sensitivity add to the difficulty of selecting indicator species. Thus, for a chemically-based system, it may be advantageous to use an insensitive species. Oysters would be better than shrimp to measure...
residue levels of DDT, because shrimp may have been eliminated from the system before monitoring could occur. By contrast, a biological program based on insensitive avian species would have failed to reveal that any problem associated with eggshell thinning existed.

Consideration of which indicator species to use also has to take into account the ecological niche of the species, especially its position in the food web, its feeding habits, and its mobility. For example, chemical analysis of clams or gulls on Lake Ontario will monitor the system in quite different ways. Clams will identify point sources of pollution, but say little about the overall pollution of the system, unless vast numbers of analyses are conducted. Conversely, gulls give an index of overall pollution, but do not indicate sources. Gulls, at a high trophic level, concentrate some compounds such as dioxins for which even low concentrations are of toxicological significance. Other pollutants such as PAH will be metabolised before they reach the higher trophic levels.

For monitoring programs to ensure environmental quality of ecosystems the structure and function of the ecosystem must be maintained and protected from the injurious effects of pollutants. Measurement of chemical residue levels is not going to ensure this. It is necessary to maintain within normal limits the structure/species composition of communities, population dynamics, and function/nutrient flow. If variations outside normal limits are observed, detailed investigations—probably both chemical and biological—are warranted to determine cause(s).

For monitoring programs to ensure the health of segments of the ecosystem, (e.g., the fishery resources of large water system), biological responses are likely to be the first approach: population size, rate of growth, reproduction, and alteration of biological markers, unless the chemicals involved are known to be few and well defined. Alterations from control values should trigger detailed studies for causes such as natural phenomena, possible over-fishing, as well as chemicals. For monitoring aimed at developing early warnings for human health, either approach (chemical or biological) could be used. The choice is likely to depend on the complexity of the chemicals involved. For example, fish carcinogen models have been developed successfully to warn of the presence of specific carcinogens in the environment.

4.5 ECOLOGICAL RESPONSES AS INDICATORS OF EXPOSURE

This section presents a brief review and analysis of the extent that ecological responses can be used as indicators of exposure. The significant impact of chemicals may not be on individual organisms; therefore, it is reasonable to also evaluate ecosystem level responses. These responses include mortality
and physiological dysfunctions in individual organisms, reproductive failure of a population, and changes in structure and ecosystem level. Thus, the frame of reference for responses of a population should be the ecosystem that contains the population. Effects that elicit a response may occur at any of these levels of organisation, and may vary in time and space; therefore, measurements of effects at any one level at any specific time yield only partial information.

Ecosystems may respond to chemical exposures by changes in structure and function. Changes in structure can be measured by determining the number and kinds of species present, and then calculating various indices, e.g., species richness (i.e., number of species per unit area), species diversity, and species abundance.

When the biota at a particular site are devastated, such changes are evident; however, subtle changes over time are more difficult to detect and evaluate. An example of the impact of chemicals on the structure of ecosystems is the research by Schindler et al. (1985) who followed the results of experimental acidification of a small lake for 8 years. Decrease in pH of the water from 6.8 to 5.0 during this period resulted in dramatic changes in the biological structure of the lake. Phytoplankton species changed and filamentous algae appeared. Cessation of fish reproduction, and disappearance and altered structure of benthic crustaceans were also noted. These changes were attributed to the hydrogen ion alone and not to secondary effects. Gray (1979) discussed the difficulty in separating pollution effects from a sewage outfall on a benthic community from natural changes, and the hazards in applying standard diversity indices to the problem. There is currently some concern about the use of species diversity indices to detect pollution induced changes in community structure and composition (SCOPE 22, 1984). A quantitative and qualitative survey of infected forest soil invertebrates illustrated the impact of SO2 emissions on infection of soil invertebrates. Other well-known structural changes that can occur from chemical exposure include changes in quality and quantity of algae near sewage outfalls due to excess nutrients, disappearance of lichens due to atmospheric pollution, and dominance of the tubificid worm Capitell capitata in some polluted areas.

It appears that response of an ecosystem to chemical exposure can be expressed by one or a few species dominating in numbers when more sensitive species are eliminated. In other instances, the most sensitive indicator could be the loss of desired species—e.g., the loss of a food chain species desirable for commercial fish or shellfish.

Biological or structural integrity of a system is important, because ecosystems are more than assemblages of organisms. They include organisms interacting with each other and the physical-chemical aspects of their environment. Indices such as general trophic composition can reveal how chemicals affect one component of a system; but the response may become
evident in a far removed compartment. Application of the organophosphate insecticide Azodrin adversely affected bollworm predators and heavy spraying of the insecticide on cotton resulted in an increase in crop damage from the bollworm. Functional aspects included such vital processes as cycling of nutrients and primary and secondary production. Likens et al. (1970) showed how clear cutting and herbicide treatment can result in the loss of nutrients to a forest system. Microorganisms and other decomposers play an important role in that they make vital materials available to consumer organisms. The toxicity of metals causing blockage of litter decomposition and subsequent adverse impact on the forest has been reported in SCOPE 22 (1984).

Ecotoxicological approaches need to be developed to measure the exposure of chemicals in the environment. For example, classical measurements of toxicity and concentration of a chemical in the water, sediment, and biota of a body of water do not necessarily yield information required to relate environmental concentrations of a chemical to exposure and of exposure to effects. Indeed, when exposure is due to a complex mixture of chemicals, and concentrations vary in time and space, the determination of exposure and the relation of exposure to effects are more complicated and, in a practical sense, impossible to associate with cause. Often, communities are too poorly understood to study directly and to determine if impairment has occurred. With the ecosystem as indicator of exposure, open-top chambers can be used to measure the reduction in growth of a plant exposed to a mixture of pollutants in the atmosphere. Two recent developments in ecotoxicological methods to evaluate exposure of aquatic communities to complex mixtures of chemicals will be discussed as examples of the kinds of tests now available.

The concept of ambient toxicity is applied to the impact of chemical effluents in receiving waters where measurements of toxicity are made without attempting to identify the toxicants. This method addresses the difficult issue of delineation of exposure that occurs in receiving waters. Ambient toxicity testing has employed cladoceran, Ceriodaphnia reticulata, and the fathead minnow, Pimephales promelas, to evaluate the impact of chemical pollutants on several streams and rivers (Mount and Norberg-King, 1985). Briefly, the method involves collecting water (usually a 24-hour composite sample) from several sites of interest in a river or stream. The cladocerans or the 0 to 2-hour old fish larvae maintained in a mobile toxicology laboratory on-site are exposed for seven days, and growth is compared with that of control solutions. It is possible, for example, by measuring chronic toxicity in this manner, that toxicity of receiving water can be assessed without using an application factor. In these tests, the conventional view of a “control” must be changed as one never knows if a chosen “control” site is free of toxicity. A pseudo-control is conducted by using some other water in which performance of the test organism is known. These results are expressed as relative toxicity rather than absolute toxicity.
Studies to date show a significant correlation between chronic toxicity values obtained with the two test organisms and instream biological measurements such as community loss index which in this case is the number of zooplankton, benthic macroinvertebrates, and fish taxa present.

The second approach is concerned with developing ecological indices that could reflect an exposure that occurs in the field. In this instance, it is more important to have laboratory test systems that represent the potential sites of impact in the field and to have some quantitative information on that representation. Effects of a known exposure of a complex mixture of chemicals associated with drilling fluids to an experimental seagrass (*Thalassia testudinum*) community was evaluated by using laboratory microcosms. One of the objectives of the study was to develop criteria for assessing effects on the community. Criteria emerging as useful in the assessment included structure of the macro-invertebrate assemblage, growth and chlorophyll content of the grass and associated epiphytes, and rates of decomposition of grass leaves in treated and untreated microcosms.

The indices selected revealed changes in the structure and function of the experimental ecosystem that were related to a known exposure. These indices, coupled with chemical analyses, may be used in the future to determine levels of chemical exposure of similar systems in the natural environment.

Where possible, it is important to compare results obtained in the laboratory with those obtained in the field. In those instances where predictive laboratory tests with communities are involved, the health of the test system should be compared with the health of a similar community in the field. Such comparisons made during development of the two tests described are encouraging.

**4.6 CONCLUSIONS**

(1) All biological monitoring methods available today measure internal exposure of either the implicated chemical/metabolite or the biological effect induced by the chemical/metabolite. Both direct and indirect methods demonstrate an alteration of a normally occurring biological process, and these alterations may retrospectively or mechanistically be related to adverse effects of concern.

(2) Several biological tests available today do not permit the assessment of internal exposures to mixtures of chemicals except for certain structurally or biologically related groups.

(3) Some of the newer tests like DNA-adduct formation may be useful in identifying groups of humans exposed to genotoxic chemicals, and it may be possible some day to relate macromolecular adduct formation to health risks.
(4) Many biological tests for assessing exposure currently available are non-specific, and are unlikely to be useful for monitoring low-dose exposure.

(5) Under restricted exposure conditions, non-specific biological tests currently used are potentially useful in identifying groups at risk as well as in characterising the chemical agents involved.

(6) For clastogenic chemicals, the most fully developed biological method is the measurement of chromosomal aberrations in peripheral lymphocytes. Although chromosomal aberrations are not specific for the agent, they are considered to be specific for the total clastogenic effect of a mixture of chemicals which, in turn, may be related to a carcinogenic risk.

(7) Knowledge and use of toxicokinetics is important in quantifying biological exposure for most substances (the four W's are crucial: why, where, when, and what to quantify).

(8) Certain information, such as environmental exposure of substances under investigation, is a prerequisite and should be made available prior to the initiation of biological monitoring.

(9) Quality control, including good analytical and statistical methods, must be an integral part of biological monitoring programs.

(10) The field of biological markers is rapidly developing, and is highly applicable to chemical exposure assessment. These markers have many advantages regarding research and control of diseases. They can certainly strengthen disciplines such as epidemiology, toxicology, preventive and occupational medicine, and risk assessment. Likewise, biological markers have ethical, legal, evaluation, and persistence limitations which should be addressed before beginning a human biological monitoring program. Criteria such as cost, invasiveness, reproducibility, variation, and validation need to be specified to justify research and application of the findings.

4.7 RECOMMENDATIONS

(1) Intensive research is required to enable calculation of test results based on biological effects from internal exposure to chemicals.

(2) In any study using non-specific biological markers, every care should be taken to account for confounding factors in its design.

(3) The predictive capability of the biological tests used in assessing health risk should be investigated.

(4) The possibility of using newly developed DNA-recombinant technology should be explored to assess exposures to genotoxic chemicals.

(5) Research aimed at determining the chemical form in which metals are present in biological materials, especially those of interest for biological monitoring, is strongly encouraged.

(6) The development, calibration, and validation of novel methods to
measure exposures to metals and other compounds in human tissues in vivo (e.g., X-ray fluorescence and neutron activation techniques) need to be encouraged.

(7) Further knowledge should be obtained about biologically reactive chemical intermediates, particularly their possible use in biological monitoring of exposures.

(8) In view of the need for studies of the role of chemical exposure in chronic diseases and of the technical difficulties of such studies, priority should be given to the search for, and the development of, biological markers reflecting integrated doses of long durations of exposure. This objective will require greater emphasis on the use of specimen banks, clinical or autopsy samples, in vivo methods for analysing toxicant concentrations, and retrospective study designs such as case-control and nested-case-control approaches.

(9) Data from different studies should be collected and banked, and access to essential data on local, national, and international levels (e.g., international registry) should be facilitated.

(10) An international bank should be established to collect biological material from identified exposures for comparative research studies and exposure registries.

(11) A coordinated program of biological monitoring is required to integrate local, national and international efforts. Ample opportunities for communication regarding successes and failures will be important in validating proposed markers.

(12) Toxicokinetic data (absorption, distribution, biotransformation, excretion, and target organ occurrence) should be collected on chemicals of importance depending on a priority list reflecting local and national needs.

(13) The inter-laboratory quality assurance (QA) program needs to be expanded, and all biological monitoring research should be integrated into a comprehensive QA network, whenever possible.

(14) Stored samples of biological materials including samples from research cohorts, routine population surveys, clinical studies, and autopsies are extremely valuable assets for biological research. In particular, older samples and those obtained from tissues allowing dose-integrating measurements are critical to epidemiological research on chronic effects. Banks of specimens from specific exposed cohorts should be developed, whenever feasible. Sample storage, contamination, and stability questions must be addressed. Gathering and use of samples requires prior consideration of the peculiar ethical and legal responsibilities involved.

(15) Biological marker protocols should explicitly address the means by which subjects will be informed of their results, the interpretation that will be given to each potential result, the type of information to be released to all concerned parties, the availability of follow-up examinations that might be recommended for individuals, and the potential legal responsibilities of
various parties. It is understood that legal questions will vary greatly among countries.

(16) Ecological responses should be the object of investigations of environment problems caused by chemicals. Specific recommendations for such investigations will depend on the ecosystem and the chemicals to be investigated. To be successful, it is necessary to have a basic understanding of an ecosystem. Capabilities must be improved to extrapolate from short-term results to long-term situations and to extrapolate from results of controlled test conditions of the laboratory to more complex field situations.

4.8 REFERENCES


