

Biological monitoring today and tomorrow

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AITIO A. Biological monitoring today and tomorrow. *Scand J Work Environ Health* 1994;20 special issue:46—58. Biological monitoring is important in the assessment of both occupational exposure to chemical agents and the health risks involved. For some chemicals data from biological monitoring can be reliably translated into health risks, while for the majority it only indicates uptake. Several workplace chemicals can be analyzed from biological specimens, but no interpretation of health risk or exposure is possible. For such chemicals, comparing the results to data from similar industries may help to determine whether smaller levels of exposure are possible. The biggest problem with most biological monitoring is that no good interpretation is immediately available. This statement is equally true for classical specific analytical chemical analyses, nonspecific assays of exposure, and almost all effect monitoring. Health-based interpretation of biological monitoring will never become possible unless biological monitoring is performed (even in the absence of immediate interpretation) and the results are stored for future analysis in a retrievable and usable form.

KEY TERMS — biomonitoring action levels, quality assurance, reference values, review.

Biological monitoring has been defined as the systematic or repetitive measurement and assessment of agents or their metabolites either in tissues, secretata, excreta, expired air, or any combination of these media to evaluate exposure and health risk, in comparison with an appropriate reference (slightly modified from reference 1). Biological monitoring has long traditions. Elevated urinary lead concentrations were reported already in the 1920s, and the first evaluations of lead in blood and urine in diagnostics and occupational health care were published more than 60 years ago (2—5). Biological monitoring has seen a rapid expansion in the last few years, from the measurement of lead, mercury, cadmium, phenol, and hippuric acid to measurements of more than 100 chemicals or groups of related chemicals (6). Biological monitoring has also been included in national and European guidelines and legislation on exposure assessment (7—16).

The key problem areas in biological monitoring remain the same: the validation of the individual measurements, quality assurance of analysis, and the ethical questions (not discussed) related to data obtained on individual workers.

In this presentation, I review some important aspects of biological monitoring from the point of view of its application in the routine of occupational health care. Other important uses and reasons for biological monitoring — such as their application in epidemiologic studies and ad hoc studies of exposure

to a chemical at a workplace without the purpose of continuous and repetitive routine — are excluded.

Purpose and scope of biological monitoring

Biological monitoring aims at preventing chemically induced health effects by providing a basis for assessing the uptake of chemicals at the workplace and the related risk. It is thus used in combination with industrial hygiene measurements, which are the traditional approach for estimating occupational exposure to chemicals. For most chemicals, industrial hygiene measurements are the best, and often the only, way of assessing exposure. However, concentrations of chemicals in workplace air are seldom stable; instead they fluctuate with time and are different in different locations. The work load dramatically changes the inhaled volume of air, and for many chemicals the amount absorbed is directly related to the amount inhaled. One should also be aware of the possibility of coincident high-exposure peaks and the increased work loads that are likely to be produced, for example, when a closed (and presumably safe) process malfunctions. Many chemicals are effectively absorbed through the skin, and this route of exposure is generally not related to concentrations of chemicals in the air. The particle size of dusts profoundly affects their deposition pattern in the airways and subsequent absorption. Personal work habits vary, and workers may absorb different amounts of chemicals under apparently similar conditions. Traditional industrial hygiene measurements do not indicate the degree of protection afforded by masks since the protection varies according to the chemicals present, the condition of the masks, and the individ-

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ual who wears it. Biological monitoring may help estimate the accumulation of chemicals in the body. This knowledge is especially important when exposure is not constant and continuous and collecting representative air samples is therefore very difficult (17).

Interpretation of biological monitoring results

Results from biomonitoring analyses are interpreted by comparing them with reference limits. Two kinds of reference limits are used in biological monitoring, reference intervals derived from unexposed populations and biomonitoring action levels.

Reference intervals for occupationally unexposed populations

Reference intervals in occupationally unexposed populations are based upon an appropriately specified model of distribution (18), derived from analytical results (*reference values*) for a sufficiently large, defined sample (*reference sample*) of a population (*reference population*). Except for the occupational exposure in question, the members of this population are similar (in terms of ethnicity, gender, age, environmental exposures due to dietary factors, air pollution, smoking, other social habits, etc) to the occupationally exposed population. For the purpose of biological monitoring, the lower limit of the reference interval is not interesting. Toxicity, rather than deficiency, is studied, and therefore the used analytical methods rarely have a sufficiently low detection limit to quantitate very low concentrations. Therefore, in biological monitoring, the term reference interval is seldom used and may not even be understood by occupational hygienists and physicians. Instead, interest is focused on its upper boundary, the *upper reference limit (URL)*. For many toxic industrial chemicals, the upper reference limit is equal de facto to the limit of detection of the relevant analytical method. Persons with measured concentrations higher than the upper reference limit are considered occupationally exposed.

In recent years, biological monitoring has been increasingly used also to explore the occupational exposure of groups of workers. It is apparent that in order to characterize the exposure of groups rather than that of individuals, a simple comparison to the upper reference limit with its given confidence limits is not appropriate. Instead, appropriate statistical techniques should be used to compare the distributions of the analytical results obtained in the allegedly exposed group with the distribution of the reference values. As a prerequisite for this approach, laboratories which perform biomonitoring analyses should provide proper information on the distribution of the reference values for unexposed subjects (ie, the number of data points and the form and parameters of the distribution). As a rule of thumb, if

all workers in a group have a biomarker level greater than half the upper reference limit, the group has probably been exposed occupationally.

Because of different environmental settings, upper reference limits vary from one geographic location to another. This variation has been extensively documented for lead and cadmium (19, 20). Moreover, changes can occur with time, as seen, for example, in the concentrations of lead in blood in several countries in recent years (21–24), and personal habits, notably smoking, are known to influence the concentrations of exogenous chemicals in body fluids, such as cadmium and benzene (20, 25). Thus upper reference limits should be determined for each geographic area, and the concomitant environmental nonoccupational exposures should be taken into account.

Often the samples for deriving upper reference limits have been collected ad hoc for assessing the occupational exposure of a group of workers, their number is small, and the sampling of the population is inadequate. Therefore, an analysis of the distribution and the subsequent setting of a reference interval cannot be done adequately. It is apparent that in the realm of generating adequate information on reference values for unexposed populations and developing reference limits biological monitoring has to and will be improved in the near future. Examples are already available (26–28).

Biomonitoring action levels for occupationally exposed populations

The second set of reference limits is based on judgments of what is acceptable exposure and what is not. These limits are thus equivalent to standards and guidelines given for concentrations of chemicals in the workplace air (ie, occupational exposure limits), and they are not based on observed concentrations in a population. For these limits, the term *biomonitoring action level (BAL)* has been proposed (29). A biomonitoring action level can be developed in the following two ways: (i) from clinical, epidemiologic, and toxicologic studies on the relationship between a measured concentration of a chemical or metabolite in body fluids and the health outcome (directly health-based biomonitoring action levels) and (ii) from experience with good work practices.

Directly health-based biomonitoring action levels are ideally derived from long-term follow-up studies of workers exposed 8 h a day, 5 d a week, over their worklife as limits below which no adverse effects on health have been observed. This is the way in which the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area defines their biological tolerance values (*Biologische Arbeitsstoff-Toleranz-Werte* or BAT) (11). Obviously, such values are not easy to obtain with confidence. The best-validated biomonitoring action levels are probably now available for lead,

cadmium, mercury, and carbon monoxide in blood and cadmium, fluoride, and mercury in urine. Directly health-based limits usually relate to a specific toxic end point and are limited by the available information; thus they may change when further data accumulate. For example, the biomonitoring action levels used in most countries for urinary and blood cadmium are based on well-defined measures of the nephrotoxicity of cadmium (30). The fact that cadmium is now considered to be a human carcinogen (31) may well lead to changes in the limits for cadmium.

Biomonitoring action levels set from experience with good work practice are usually derived mathematically from occupational exposure limits as the concentration of the relevant chemical or metabolite that will occur in biological material from an average worker after an 8-h time-weighted-average exposure at the level of the prevailing occupational exposure limit. This is the way in which the American Conference of Governmental Industrial Hygienists defines their *biological exposure indices (BEI)* (9) and the German Senate Commission defines the biological exposure equivalents for carcinogens (*Expositionsequivalente für krebserzeugende Arbeitsstoffe* or *EKA*) (11). Health protection provided by biomonitoring action levels derived from occupational exposure limits thus depends on the following factors: (i) the appropriateness of the occupational exposure limit (a compromise between health protection and cost factors), (ii) the reliability of the estimate of the relationship between the occupational exposure limit and the biomonitoring action level, (iii) and the adequacy of the sampling strategy. A biomonitoring action level derived from an occupational exposure limit for a chemical with a short half-time in the body is crucially dependent on the timing of the sample collection. The biomonitoring action level is usually the best estimate of a concentration in the biological specimen equivalent to the occupational exposure limits (from an empirical regression line which describes the relationship between concentrations in workplace air and in the biological specimen). Therefore, the actual exposure for approximately half of the group of workers is in excess of the occupational exposure limit, even when the compliance with the biomonitoring action level is notionally 100%. However, most workplace standards aim at protecting "most workers" (9, 11). Hence the biomonitoring action level and occupational exposure limits are not commensurate. Furthermore, the uncertainty around the estimate varies widely between different chemicals. For example, after exposure to styrene, the uncertainty is small for mandelic acid or mandelic acid plus phenylglyoxylic acid in urine (for which a large number of studies with concordant results have been published), but large for styrene itself in urine (for which few studies have been published) (32). An important problem for biomonitoring action levels that are based on occupational exposure limits is that no single biomonitoring action level can

be given for different compounds of an element. The relationship between the airborne concentration of, for example, readily water-soluble nickel compounds and the urinary nickel concentration differs very much from that for the practically insoluble nickel oxides, sulfides, and metallic nickel (33). The same is true for cobalt (34) and chromium (35).

For chemicals that are extensively absorbed through the skin, biomonitoring action levels cannot be derived from the occupational exposure limits. In such cases the only way of determining a biomonitoring action level is to use the purely pragmatic process of defining what concentrations of a chemical or metabolite in biological specimens occur in exposed workers when good work practices are adhered to. Thus a biomonitoring action level may, for example, be set at a value that is 90% of the level the workplaces have actually achieved. The British Health and Safety Executive and the Finnish Institute of Occupational Health have used this method, for example, to set the biomonitoring action level for methylenebis(2-chloroaniline) (MOCA) (10, 36).

The main task of research into biological monitoring is clearly the development of biomonitoring action levels (ie, the validation of biological monitoring methods). Although the directly health-based action levels are the most desirable, it is apparent that, for most chemicals, these remain elusive. Meanwhile, determining the relationship between exposure and the biologically observed concentration is important, and such effort should be required from every author trying to publish a paper on biological monitoring — the mere reporting of a correlation coefficient is useless. One requirement for all laboratories doing biological monitoring is that they advise the occupational health services using these analyses of how each reference interval and biomonitoring action level was derived and what the confidence limits are.

Approaches in biological monitoring

Biological monitoring has traditionally been divided into two branches, the biological monitoring of exposure and the biological monitoring of effect (37). The former means measurement of the parent chemical or its metabolite in biological samples, and the latter is, ideally, the measurement of an "nonadverse" or "reversible" effect of the chemical on the body.

Biological monitoring of exposure

The term *monitoring of exposure* — like the equivalent biomarker of exposure — is a misnomer, since the purpose is not to measure exposure (contact of the body with the chemical) but the amount absorbed. However, the term is in wide and expanding use (37), and it is probably not wise to add to the confusion by proposing a change. The purpose of this activity is to measure the concentration of the chemical or

its metabolite in the body fluids or excreta as opposed to its effects on the body. This concentration is then assessed in a comparison with reference values so that occupationally exposed persons can be identified and the exposure or uptake can be quantitated or the magnitude of the health risk caused by the exposure can be directly estimated. (See the preceding section on interpreting results). The most studied biological monitoring methods make use of selective chemical methods; recently, several nonselective methods have also been suggested.

Selective methods. Methods that are selective intend to measure exclusively the object of the biological monitoring program. The selectivity depends on analytical specificity, but in situations in which a metabolite — rather than the chemical itself — is measured, there is also dependence on sources of the metabolite other than the occupational exposure.

Analytical specificity is the capability of the method to measure exclusively the chemical wanted, namely, the analyte. The specificities of different analytical methods vary widely. Typically photometric methods are less specific than methods using chromatographic separation or atomic absorption. Modern techniques employing reasonably specific detection and efficient sample purification by chromatographic methods in combination are often very specific. However, specificity depends on the presence of disturbing chemicals and is therefore very much dependent on the matrix. A method that is fully specific in one matrix may be nonspecific in another.

The specificity of the parameter being measured with regard to the occupational exposure is an important consideration. For example, varying amounts of phenol, hippuric acid, and cresols are derived from dietary components and are excreted in urine, as is arsenic if it is measured as total arsenic in the urine. It is therefore difficult to estimate occupational exposure to benzene, toluene, or inorganic arsenic by these analyses. The significance of this nonoccupational chemical source is inversely related to the level of occupational exposure. At high exposure levels, urinary concentrations of these metabolites may accurately reflect the occupational exposure, while at low levels of occupational exposure such measurements may be completely useless and misleading. All of the cited measurements are easier to carry out than the corresponding specific analyses (blood benzene, blood toluene, urinary inorganic arsenic). It is therefore important to study the exposure levels to be encountered — and accepted — before starting a biological monitoring program. The parameter to be measured should be the one that is optimal under the local conditions.

A similar problem of specificity can be encountered when unchanged elements or, for example, long-lived environmental contaminants such as chlorinated pesticides are measured. Biological monitor-

ing measures the chemical independent of whether it is derived from the occupational or environmental exposure. From the point of view of the health risk, this is a marked strength of biological monitoring in that the chemical has identical effects on the body independent of the source. However, from the point of view of prevention, this is a marked weakness of biological monitoring in that the source of the potentially harmful exposure has to be clarified by other means.

Still another consideration of selectivity in the biological monitoring of mainly inorganic chemicals is speciation. With the exception of arsenic and mercury, biological monitoring has only concerned analysis of the total amount of an element present in the biological fluid without making any separation by, for example, valence states, or the difference between ionized and nonionized species. When the chemical has a systemic effect (lead, mercury, cadmium, aluminum), this is no problem, but for chemicals such as nickel and nickel compounds and chromium and chromium compounds, for which the important health effects are mainly local (skin, respiratory tract) and there are remarkable differences in the toxicity and kinetics between different species of the element, this is an important limitation in biological monitoring. Although there have been some efforts (38, 39) to produce a rough speciation of chromium in biological fluids, as a generic problem in biological monitoring, this question seems unanswerable at present.

Macromolecule adducts. During the last few years, the analysis of macromolecule — protein or DNA (deoxyribonucleic acid) — adducts has been extensively studied with the purpose of developing biological monitoring methods (40). Among the most extensively studied chemicals or groups of chemicals have been aromatic amines and polycyclic aromatic hydrocarbons (PAH) (41–45). The idea is that the dose of the proximate carcinogen is measured at the target molecule [DNA adducts or their surrogates = protein adducts] — albeit usually not in the target cells — and this measurement could reflect the health risk better than, for example, the concentration of the parent chemical in the blood stream. This has been a very promising approach for some 15 years, but it is still plagued by the same problems: no quantitative validation based on health outcome or exposure is available and the techniques used are very sophisticated and still tend to be working at their limit of detection when samples from occupationally exposed people are analyzed.

Nonselective methods. An early representative of nonselective methods is the iodine azide test that was used to monitor carbon disulfide biologically (46, 47); currently this method has only historical inter-

est. Nowadays nonselective methods are mainly used to monitor chemicals that are (or are transformed in the body into) electrophilic reactants, and the results of such a reaction are monitored, usually in the urine.

Urinary thioethers are mainly derived from the reaction of electrophilic chemicals with the tripeptide glutathione. After this alkylation reaction, glutathione thioethers are enzymatically degraded to yield N-acetyl-S-alkylcysteines, or mercapturic acids, which are excreted in the urine. Evaluating carcinogen exposure from urinary thioether excretion poses several problems. A variable amount of thioethers is derived from dietary sources (48, 49). Because of the differing chemical structure of the thioethers, their recovery in the analysis may be variable and low. Analyzing urinary thioethers has to be regarded as a signal of exposure only, and it can be used in the follow-up of exposure in selected cases. Analyzing specific thioether compounds, such as that of phenylmercapturic acid, as a means of biologically monitoring benzene (50, 51), does not belong to the class of nonselective methods. Such specific thioether analyses may well become more feasible when the use of modern, inexpensive, and user-friendly mass spectrometric detectors becomes more widespread.

Measuring mutagenicity in urine has also been used to estimate carcinogenic exposure by nonselective means (52, 53). It is based on the propensity of genotoxic chemical carcinogens, irrespective of chemical structure, to induce mutations in microorganisms. Urine poses some problems in mutagenicity testing, notably the low concentrations of mutagens in the urine, the presence of factors in the urine that either enhance or inhibit the growth of the indicator organisms used, and the presence of the mutagen in the urine in an inactive form.

Smoking is often the most formidable problem when mutagen or carcinogen exposures are being evaluated by mutagenicity assays. Tobacco smoke contains a variety of chemicals that are excreted in the urine in a form that may be converted to a mutagenic form. However, most of the mutagens in tobacco smoke are frame-shift mutagens, and in situations in which the occupational exposures contain mutagens of other types, the smoking effect can be removed if bacterial strains not sensitive to frame-shift mutagens are used.

One should not forget that only mutagenic carcinogens can be detected by mutagenicity assays. In addition, it is possible that carcinogenic chemicals are excreted in the urine in a form that is not mutagenic when analyzed by standard techniques. In addition, the concentration methods applied may show limited recovery for the mutagens in the urine. Therefore, negative results in urine mutagenicity testing should not be considered to indicate absence of exposure to carcinogenic chemicals. The analysis of mutagenicity in urine has the additional problem that it cannot readily be quantitated. Because of these problems, the number of studies on the assays of mutagenicity

as a means of exposure monitoring has decreased recently. For example, in a large symposium on biomonitoring and susceptibility markers in human cancer in the fall of 1991 in Hawaii, where some 90 studies were presented, not even one paper reported on the use of mutagenicity assays. Urinary mutagenicity monitoring has, however, proved useful in the detection and, especially, follow-up of carcinogen exposure when preventive hygienic measures have been implemented in carefully planned situations. Its primary field of application is to complex exposures, which, by prior analysis, have been shown to include mutagenic chemical species.

Biological monitoring of mixed exposures

Traditionally, biological monitoring is used to evaluate the uptake of a single chemical, and a method that is as specific as possible is used. However, very seldom are workers exposed to a single chemical, and often it would be advantageous to assess exposure to a mixture. In biological monitoring, an indicator chemical has been selected for such assessments and the chemical or its metabolite is analyzed. In such a situation one has to assume that the indicator chemical behaves in the organism in a manner similar to that of the toxicologically important component(s) of the mixture — otherwise one has to assume a stable composition for the mixture in order to be able to make any assessment of the risk. The biological monitoring of mixed exposures has been the most extensively studied in cases of PAH exposure and tobacco smoke. In addition to this "indicator chemical" approach, nonselective methods (see the preceding section) have been extensively studied as a means of biologically monitoring mixed exposures.

PAH usually occur in complex mixtures, and their main toxicologic interest is the carcinogenic activity that is most apparent in hydrocarbons with five to six fused aromatic rings, such as benzo[a]pyrene. PAH are readily absorbed through the skin. The biological monitoring of exposure to PAH has been mostly studied with analyses of 1-hydroxypyrene, a metabolite of pyrene, in the urine of exposed workers. Several investigations have shown that the concentration of 1-hydroxypyrene is elevated in occupational or experimental dietary or cutaneous PAH exposure, and also in smokers (versus nonsmokers) (54–63). The main problem of 1-hydroxypyrene measurements is that the toxicologically important PAH are not measured, and the interpretation of the result is crucially dependent on the ratio of pyrene to the carcinogenic PAH in the airborne mixture.

Passive smoking is being increasingly perceived as — also — an occupational hazard, and biological monitoring methods to identify smoke-exposed people have been developed. The most extensively used of these have been analyses of thiocyanate, thioethers and cotinine in urine, cyanide, nicotine and its metabolite cotinine in plasma, and carbon mon-

oxide or carboxyhemoglobin in blood (64–70). All of these parameters show elevated levels in people that have been exposed to cigarette smoke — or have smoked themselves. However, especially, urinary thioethers but, in some occupational settings, also carbon monoxide concentrations in blood are very nonselective. Therefore, cotinine concentrations, either in plasma or in urine, seem to be the best choice at present. Cotinine is preferable over nicotine because of its longer half-time (approximately 20 h) both in urine and in plasma.

Although some data exist on the relationship between the airborne concentration of PAH and pyrenol in urine (59, 60), it currently seems that using analyses of indicator chemicals to interpret the results of biologically monitoring mixed exposures is at best semiquantitative. Exposed groups may be identified, but it is not possible to set biological action levels that are based on health risk or industrial hygiene standards.

Assessment of body or organ burden in vivo

Most chemicals are distributed into several different compartments of the body. For several, a tissue or organ can contain considerable concentrations (ie, a chemical is stored there). Thus, for example, lipid-soluble solvents are accumulated in body fat, cadmium in the liver and kidneys, and lead in the bones. Recently methods have been developed to estimate directly the amount of chemical at the site of accumulation. The most widely used methods are neutron-activation measurement for cadmium and X-ray fluorescence analysis for lead and cadmium.

Neutron activation. Cadmium content is sometimes measured by neutron activation in the liver and kidneys, sites which show marked accumulation (20). The sensitivity of the assay depends on the dose of radiation used. Levels occurring in the liver and kidneys of occupationally unexposed adults can be measured, and the accumulation of cadmium in the kidneys of smokers has, for example, been demonstrated and quantitated using this technique (71). However, although neutron activation *in vivo* would be an effective tool in predicting the risk of kidney damage in cadmium exposure, it involves exposure to neutrons, which are still a rather unknown entity. The use of this method in routine biological monitoring would seem questionable.

X-ray fluorescence. The lead content of bones has been measured by X-ray fluorescence, as has the content of cadmium in kidney (72, 73). If polarized X rays are used, the sensitivity of the method for lead analysis is currently sufficient to measure the lead concentrations in the bones of preschoolchildren (74). The kinetics of lead in the organism during and after occupational exposure has been elucidated ef-

fectively with this method (72, 73, 75). The radiation dose is very small, and only small volumes of tissue are irradiated. However, it seems that a good history of the blood lead concentration reflects the effects of lead on neurophysiological and psychological parameters better than the content of lead in the tibia (Mannelin et al, unpublished findings). Thus the main area of application for the determination of lead in bone seems to be in cases in which such a history is not available and there is suspicion of lead-induced health impairment (ie, in diagnostics rather than in biological monitoring).

In vivo methods have also been proposed for the analysis of mercury (76, 77), aluminum (78), iron (79), and silicon (80). However, only in the case of cadmium analysis in the kidney and liver is it at present readily apparent that the analysis of body burden enhances the power of traditional biological monitoring relying on the analysis of body fluids. For no other measures is the relationship between the measured quantity and the health effect known. In addition, for analyses of nontarget tissues (such as bone for lead, aluminum, or mercury), it is by no means clear that the relationship between the concentration there and that in the target tissue — or the effect on the target tissue — is any closer than that between the concentration in blood or urine and in the target tissue. Therefore, the basic problem of biological monitoring appears once again, the validation is lacking.

Biological monitoring of effects

Effect monitoring means that, instead of the substance itself or its metabolite being measured, some specific or semispecific effect is determined. Monitoring an early effect of a chemical would, in a way, be ideal for preventing the adverse health effects of a chemical. It would even take into account the individual differences in susceptibility, not only the amounts of the chemical taken up. In order to prevent adverse effects, the effect on which the monitoring is based should be reversible and nonadverse, and it should predict the adverse effects.

Traditional toxicity end points. Effects that have been extensively used in biological monitoring programs include carboxyhemoglobin content of the erythrocytes, plasma and blood cholinesterase activities, enzymes and intermediate levels of hemoglobin synthesis, and proteins or enzymes in urine.

The analysis of carboxyhemoglobin is on the borderline between effect and exposure monitoring. It is a well validated biomarker. The levels at which health effects can be expected in healthy people and in persons with cardiovascular disease are well established (81). However, this analysis has the problem common to all biomarkers of chemicals with acute (only) effects. If the worker survives (or if he does not) until the sample is collected and the anal-

ysis is done and the results are made available to the health care personnel, he will not probably gain anything from the result. That is, monitoring chemicals with only acute effects cannot be effectively preventive. — It is of course true that the measurement may still be useful in characterizing the exposure situation (ie, in indicating that there is high, low, or no exposure to carbon monoxide). Carboxyhemoglobin analysis differs from all other effect monitoring methods because it is specific for exposure. Elevated levels can only be induced by exposure to carbon monoxide (or dichloromethane, which is metabolized to carbon monoxide). On the other hand, carboxyhemoglobin is not specific of occupational exposure. Exposure from tobacco smoking is much more common than occupational exposure as a cause of elevated blood carboxyhemoglobin levels.

The analysis of acetyl cholinesterase activity in erythrocytes or that of pseudo cholinesterase in plasma among workers exposed to organophosphate (or carbamate) insecticides has been well validated in the diagnosis and treatment of high exposure. However, the relationship between these enzyme activities and the symptoms is not equally close at low or moderate exposure levels. For assessing low exposures, the analysis of urinary alkyl phosphates has been proposed (82). However, especially in developing countries, where organophosphate poisoning is still common, cholinesterase inhibition remains the first choice for the biological monitoring of organophosphorus pesticides.

The most cherished example of effect monitoring is the assessment of exposure to lead from its effects on the enzymes of hemoglobin synthesis, such as the 5-aminolevulinate dehydratase or ferrochelatase, or the concentrations of different intermediates in this synthetic pathway, such as zinc protoporphyrin in erythrocytes or aminolevulinate in urine. Where the analysis of lead can be performed reliably, these measures do not offer additional information in the biological monitoring of lead exposure. It has not been demonstrated that persons who show changes in hemoglobin synthesis at lower-than-average levels of lead exposure are exceptionally sensitive to the effects of lead on the nervous system, and these effects are the critical ones at current levels of exposure to lead. Thus lead-induced health effects cannot be prevented more effectively by following these effect monitors than by analyzing lead in blood — and keeping the levels low enough. Furthermore, it has been proposed that these effects are not really “nonadverse.” For example, the reserve capacity for blood formation required after blood loss was decreased in people with such subtle lead effects (83).

The situation is different in developing countries, where, for example, analyzing the erythrocyte protoporphyrin could be a cost-effective way of monitoring lead exposure. The instrumentation is inexpensive, contamination in sample collection is not a problem, the analysis is very simple, reasonable ac-

curacy is easily achieved, and the limited specificity of the assay could turn out to be an advantage. People with iron deficiency anemia would also be picked up by the biological monitoring program.

The excretion of a variety of proteins and other compounds has been investigated as effect monitors of chemicals that are toxic to the kidney, such as mercury, lead, and, especially, cadmium (30, 84, 85). In the studies, none of these agents were considered useful for the biological monitoring of lead or mercury. However, more than 10 parameters were found to correlate with long-term exposure to cadmium, and several could be used for the biological monitoring of this nephrotoxic chemical. Of the 10, beta₂-microglobulin and retinol-binding globulin have been the most extensively studied. However, elevated excretion of these markers in the urine is not reversible (with the possible exception of a very minor elevation). Cadmium exposure that causes such a change will later lead to indisputable kidney damage such as an accelerated age-dependent decrease in the glomerular filtration rate (86—90). In addition, since the prediction of health effects from the urinary levels of cadmium is reliable, it is not clear that effect monitoring can add any further insight to the biological monitoring of cadmium. Again, the situation is probably different in areas in which urinary cadmium cannot be analyzed reliably. In such circumstances, analyzing retinol binding globulin (beta₂-microglobulin is notoriously unstable in acid urine) is an alternative worth considering, although it is fairly nonspecific (91—94).

Genotoxic end points. Most proved human carcinogens are clastogenic (ie, they cause structural chromosome aberrations, micronuclei, or sister chromatid exchanges, all of which can be detected microscopically). Several of these chemicals have been shown to cause cytogenetic damage in occupationally exposed people. There is thus a rationale for using cytogenetic methods in the biological monitoring of people exposed to genotoxic carcinogens (95—97). Chromosome aberrations have the closest mechanistic links to carcinogenesis, but until recently their analysis was extremely cumbersome and tedious. However, recently developed computer programs that locate cells in the metaphase have greatly facilitated the analysis of chromosome aberrations — but still the analysis requires highly skilled expertise and is to some extent subjective. The analysis of sister chromatid exchanges is technically less demanding and less time consuming. However, the biological basis of the phenomenon is not well understood, and the circumstances of cell cultivation have a remarkable effect on the frequencies of the sister chromatid exchanges detected. The analysis of micronuclei has not been studied equally extensively, but the analysis can be automated and therefore shows promise even for routine application. When