Ann. Ist. Super. Sanità, vol. 32, n. 2 (1996), pp. 199-206

Biomonitoring and occupational medicine. Possibilities and limitations

Jürgen ANGERER and Jürgen GÜNDEL

Institut für Arbeits- und Sozialmedizin und Poliklinik für Berufskrankheiten, Universität Erlangen-Nürnberg, Erlangen, Germany

Summary. - In the prevention of occupational diseases, biological monitoring has become very important. Today the individual level of exposure to many toxic substances can be assessed by routinely monitoring their concentrations in blood and urine. The adoption of effective quality control procedures allows reliable analytical results down to the ppt range to be obtained. Limit values such as the biological tolerance values for occupational exposure (BAT) from the Deutsche Forschungsgemeinschaft in Germany and the biological exposure indices (BEI) from the US American Conference of Governmental Industrial Hygienists (ACGIH) help to interpret the analytical results of biological monitoring. In spite of numerous advancements there are some gaps in the field of biological monitoring. Some groups of substances, such as pesticides, are still beyond the possibilities of biological monitoring. With regard to biological and biochemical effect monitoring, large gaps have to be filled and considerable research is needed. The number of limit values evaluated till now is still too small. Politics, however, seems to be the most serious limitation for biological monitoring.

Key words: biological monitoring, occupational and environmental medicine, biological tolerance values, biological exposure values.

Riassunto *(Possibilità e limitazioni del monitoraggio biologico)*. - Nella prevenzione delle malattie professionali, il monitoraggio biologico è diventato molto importante. Oggi il livello individuale di esposizione a molte sostanze tossiche può essere valutato monitorandone di routine le concentrazioni nel sangue e nelle urine. L'adozione di efficaci misure di controllo di qualità fa sì che possano essere ottenuti risultati affidabili fino al livello delle ppt. Per gli indicatori biologici, valori limite quali i valori di tolleranza per l'esposizione occupazionale stabiliti dalla Deutsche Forschungsgemeinschaft in Germania (BAT) e gli indici di esposizione della US American Conference of Governmental Industrial Hygienists (BEI) sono di aiuto nell'interpretazione dei risultati analitici del monitoraggio biologico. Alcuni gruppi di sostanze, quali i pesticidi, sono tuttora al di là delle possibilità del monitoraggio biologico. Per quanto riguarda il monitoraggio di effetti biologici e biochimici, c'è ancora molto da fare ed è necessaria una considerevole attività di ricerca. Inoltre, il numero di valori limite definiti finora è ancora piuttosto basso. Tuttavia, la più seria limitazione al monitoraggio biologico è costituita da un insufficiente sostegno a livello politico.

Parole chiave: monitoraggio biologico, medicina occupazionale e ambientale, valore limite biologico, indici biologici di esposizione.

Introduction

For about 100 years the prevention of occupational diseases caused by chemical substances has been managed by the estimation of the external exposure, i.e., by measuring the concentrations of hazardous substances in the air of the work places (ambient monitoring).

Undoubtedly this strategy of measuring external exposure has been successful in the prevention of occupational diseases. In spite of this, ambient monitoring has some decisive drawbacks. It is mainly based on the assumption that: a) the chemicals are absorbed by inhalation only, and b) the concentration of chemicals measured in the air is representative of the internal dose. However, other routes of absorption are not taken into account. Moreover, with decreasing vapour pressure of the chemicals it becomes increasingly more difficult to obtain measurements which are representative of the internal exposure.

Internal exposure, which more closely reflects the absorbed dose, can be assessed by the determination of chemicals and their metabolites in biological media.

Biological and biochemical effects provide a better possibility of assessing individual health risk. An increased elimination of δ-aminolevulinic acid in the case of lead exposure is an example of a biological effect parameter. Adducts of carcinogenic substances to DNA or haemoglobin are nowadays called biochemical effect parameters. For the estimation of internal exposure and biological or biochemical effects, biological monitoring

represents a *conditio sine qua non*. The efficiency in the prevention of occupational diseases grows in the direction ambient monitoring/biological monitoring.

Zielhuis suggested the following definition for biological monitoring [1]:

Systematic continuous or repetitive activity for collection of biological samples for analysis of concentration of pollutants, metabolites or specific non-adverse biological effect parameters for immediate application, with the objective to assess exposure and health risk to exposed subjects, comparing the data observed with a reference level and - if necessary - leading to corrective actions.

This definition gives an idea of the major points under discussion today and includes the determination of chemical substances and their metabolites in human body fluids. It also includes the biological and biochemical effect monitoring.

Advantages

Compared with ambient monitoring, biological monitoring offers the advantage that dermal and oral uptake are considered. Many substances enter the body through the skin, e.g. aromatic amines and nitro compounds, organochlorine pesticides, phenols, organophosphorous ethers and thioethers. Furthermore, organic solvents, especially glycol ethers and dimethylformamide, are readily absorbed through the skin.

Oral uptake is also important in the case of exposure to dust containing toxic substances like metals. Ventilatory parameters, varying among other factors with the work load, as well as interindividual variation in the capacity to detoxify are taken into account by biological monitoring. Synergisms and antagonisms can also be detected by biological monitoring. The efficiency of protective clothing, different distances from the source of emission and personal hygiene habits have influences on the results of biological monitoring.

Requirements

To perform biological monitoring the following requirements should be fulfilled:

a) availability of suitable biological media;

b) availability of suitable and reliable analytical methods, kept under control by quality assurance and good laboratory practice;

c) availability of suitable parameters, able to reflect exposure or early non-adverse health effects;

d) availability of limit values which enable the interpretation of results. These are reference values for the general population and limit values for occupational exposure.

Biological media

Biological materials should be easily accessible in sufficient amounts and under routine conditions. The collection of the specimens should not subject the worker to any undue stress. For this reason, blood and urine are the biological media almost exclusively used for monitoring purposes. Blood, being the central compartment, is in a steady state with all organs. It is especially suitable for the detection of unchanged substances with long half lives like metals or persistent organochlorine compounds. Urine is readily accessible in large volumes, allowing the determination of low concentrations of chemicals, such as those due to environmental exposure. Urine is especially suitable for the determination of metabolites of substances with short half lives, such as many organic solvents. The main disadvantage of using blood for biological monitoring is the limited sample volume available. On the other hand, urine dilution is variable, therefore requiring the collection of 24 h samples or standardization with creatinine.

Parameters

The parameters used for the estimation of internal exposure and health risks should sensitively and specifically indicate the exposure to a given substance. Furthermore the parameter should reflect the toxic effect as close as possible. The benzene content in blood for example is the most sensitive and yet specific parameter for benzene exposure down to the concentration range caused by environmental exposure. Using dynamic headspace analysis it is possible to separate non smokers, passive smokers and active smokers from one another using benzene blood levels as a criterion. The unchanged benzene molecule, however, is relatively far away from the effect. In the case of benzene leukaemia, the determination of t,t,-muconic acid, S-phenylmercapturic acid or adducts of benzene to proteins or DNA are more closely related to the biological response.

Today the possibilities for biological monitoring can roughly be categorized according to specificity and sensitivity. Specificity must be subdivided into specificity for the substance and specificity for its effect. The determination of the unchanged substance in body fluids is of course highly specific for exposure to a given substance, but in many cases unspecific for the effect. Metabolites in body fluids take a medium position being more or less specific for substance and effect. The sensitivity of the detection of chemicals and their metabolites in biological media is, however, unsurpassed by all other possibilities of biological monitoring. Biological effect parameters, like δ-aminolevulinic acid, cholinesterase, zinc protoporphyrin, and biochemical effect parameters, like adducts to proteins or to DNA, are

almost ideal parameters for biological monitoring. These parameters are both specific for the substance and for the effect. However, from a diagnostic point of view, they do not indicate internal exposure as sensitively as the determination of concentrations of chemicals in blood or urine. Cytogenetic parameters, such as sister chromatid exchanges, micronuclei and chromosomal aberrations, are highly specific for the effect but are not specific with regard to the exposure to a given chemical. The same is true for tests on mutagenicity in urine which, moreover, are rather insensitive. When cytogenetic parameters, mutagenicity tests or certain metabolites are used for biological monitoring or health surveillance, the unchanged substance should be determined in parallel. Only in this way the observed effect can be traced back to a particular exposure: a particular substance can only be held responsible for adverse health effects if at least one of the parameter used for biological monitoring is a specific indicator for that substance.

Table 1 shows an overview of the substances which can be routinely determined in biological materials in our laboratories. This list contains practically all metals which are of occupational and environmental interest, from aluminium to zinc. These substances can be determined in both blood and urine. As already mentioned, urine offers the advantage that lower detection limits can be obtained. Among the organic substances the solvents and their metabolites play a major role in occupational and environmental medicine. Organochlorine compounds are important from an environmental point of view and in specific work places, such as those where decontamination of polluted soil and combustion processes, like the incineration of municipal waste, take place [2].

Analytical methods

The reliability of analytical results is an essential requirement of biological monitoring. Only results which bear comparison among themselves, with different laboratories and with limit values lead to a reliable assessment of risk. For this purpose reliable analytical methods are necessary. Some national organizations such as the Deutsche Forschungsgemeinschaft (DFG) in Germany, and the National Institute for Occupational Safety and Health (NIOSH) in the USA describe analytical methods which are controlled for reliability and reproducibility. These methods are published in a form which can be used as a guide for the technician performing the analyses. These so-called standard operating procedures (SOP) fulfil the requirements of good laboratory practice (GLP) and are valuable contributions to the comparability and accuracy of analytical results. In general, such methods are published by the DFG in *Analyses of hazardous substances in biological materials* [3]. Additionally the application of these methods must be tested by internal and external quality assurance [4-7].

Instrumental analysis offers us today the opportunity of determining the internal exposure to practically all substances taken up in the human body. The state of the art is represented by the determination of polychlorinated dibenzodioxins as an example for organic substances and platinum as an example for inorganic substances: these chemicals can be determined in body fluids down to concentrations of 1 pg/l [8] and 1 ng/l [9], respectively.

Limit values

For the interpretation of results obtained from biological monitoring, reference and tolerance values are necessary. Reference values have been usually defined as the 95th percentile with regard to the range of concentration of a given parameter in body fluids of subjects not occupationally exposed. International and national working groups are currently discussing the question of setting reference values. For many hazardous substances like metals, organochlorine compounds, organic solvents, there is a lot of material which enables the evaluation of reference values. Table 2 reports the reference values accepted in Germany for some chemicals which are relevant to environmental and occupational health. These reference values are important for occupational medicine because with their help it is possible to decide whether there is any exposure at all at the workplace.

However, for occupational health purposes, tolerance values are of even greater importance. In 1979 the DFG Commission for the investigation of health hazards due to chemical compounds in the work area started to evaluate such values called "Biological tolerance values for occupational exposure" (BAT-values) [10]. The list of the BAT-values is increased every year. In 1985, a working group of the American Threshold Limit Values (TLV) Committee also began to set such standards called "Biological exposure indices" (BEI-values) [11]. The BAT-values for organic solvents established in Germany are reported in Table 3.

The BAT-values are defined as the maximum permissible quantity of a chemical compound, its metabolite or any other deviation from the norm of biological parameters which do not impair the health of the employee. BAT-values are ceiling values for healthy individuals. Ideally BAT-values are evaluated by correlation between external exposure, internal exposure and health effect. At exposures up to the BAT-values there should be by definition:

a) no impairment of functional capacity;

b) no decrease in the ability to compensate additional stress;

c) no decrease in the ability to maintain homeostasis;

d) no enhancement of the susceptibility to other environmental influences;

e) all effects, if any are present, must be reversible.

Table 1. - Overview of the substances which can be determined in biological materials routinely in our laboratory

Metals and relevant indicators of the human metabolism	Blood	Serum/ Plasma	Urine
Albumin			•
Aluminium		•	•
Antimony			•
Arsenic			•
δ-Aminolevulinic acid			•
Barium			•
Beryllium			•
Cadmium	•		•
Calcium		•	
Chromium	•	•	•
Cobalt	•		•
Copper		•	•
Iron		•	•
Lead	•		•
Manganese	•	•	•
Mercury	•		•
β_2-Microglobulin		•	•
Molybdenum		•	•
Nickel	•		•
Potassium		•	
Selenium	•	•	
Sodium		•	
Tellurium			•
Thallium			•
Total protein			•
Vanadium			•
Zinc		•	•

Organic solvents and their metabolites			
Acetone		•	•
Aliphatic hydrocarbons-screening	•		
Aromatic hydrocarbons-screening	•		
Benzene environment	•		
Benzene worplace	•		
Butanol	•		
Butanone (Methylethylketon)			•
Butoxy acetic acid			•
Butylacetate (as butanol)	•		
Carbondisulfide (as 2-thio-1,3-thiazolidine-4-carboxylic acid)			•
Chlorobenzenes	•		
Cyclohexanone	•		
Dichloroethanes	•		
Dichlororethenes	•		
Dichloromethane	•		
Dichloropropane	•		
Dimethylphenols			•
Dioxane	•		
Ethanol	•		
Ethoxy acetic acid			•
Ethylbenzene	•		
Formic acid			•
Frigene	•		
Halogenated hydrocarbons-screening	•		
Heptanone	•		
Hexane	•		
Hexandione			•
Hexanol	•		

Organic solvents and their metabolits			
Hexanone	•		
Hippuric acid			•
Isopropanol (as acetone)			•
Mandelic acid			•
Methanol			•
1-Methoxypropanol	•		•
Methoxy acetic acid			•
Methylhippuric acids			•
Methylacetate (as methanol)			•
Methylisobutylketone	•		
Methylphenol			•
t,t-Muconic acid			•
Phenol			•
Phenylglyoxylic acid			•
Propanol			•
Styrene	•		
Tetrachloromethane	•		
Tetrachloroethane	•		
Tetrachloroethene (Perchloroethene)	•		
Toluene	•		
Toluric acids (Methylhippuric acids)			•
Trichloroethane	•		
Trichloroethene	•		
Trichloromethane	•		
Trichloroacetic acid			•
Trichloroethanol	•		
Xylenes	•		

Aromatic and aliphatic amines, nitrocompounds and their metabolites			
Aromatic amines-screening			•
Aminophenol			•
Aniline			•
Diaminodiphenylmethane			•
Dichlorobenzidine			•
4,4'-Methylene-bis(2-chloroaniline) (MOCA)			•
Nitro aromatic compounds	•		
Toluylenediamine			•

Pesticides and their metabolites			
Chlorophenols		•	
Cholinesterase activity		•	
p,p'-DDE		•	
Hexachlorobenzene		•	
Hexachlorocyclohexane (HCH) (Lindane)			•
Parathion (as nitrophenol)			•
Pentachlorophenol (PCP)		•	•
Polychlorinated Biphenyls (PCB)		•	

Others			
CO-Haemoglobin	•		
Fluoride		•	•
Met-Haemoglobin	•		
1-Hydroxypyrene			•
Thiocyanate		•	•

Table 2. - Some reference values with importance to environmental and occupational health which are accepted in Germany

Substance	Specimen	Reference value (µg/l)
Inorganic substances		
Arsenic	urine	< 15
Cadmium	blood	< 1
Chromium	urine	< 1.5
Lead	blood	< 150
Mercury	urine	< 3
	blood	< 2
Nickel	urine	< 2.5
Thallium	urine	< 1
Organic substances		
Benzene	blood	< 1
Toluene	blood	< 5
Tetrachloroethene	blood	< 1
Trichloroethene	blood	< 0.3
γ-HCH	blood	< 0.1
PCP	blood	< 25
Σ PCB	blood	< 8
HCB	blood	< 4
1-Hydroxypyrene	urine	< 2

Gaps

The applications of biological monitoring seemed to be unlimited. In spite of the apparently unlimited possibilities, there are some gaps in the assessment of internal exposure to chemicals. For instance, there is a lack of analytical methods for a reliable determination of many pesticides, like pyrethroides, triazens and carbamates, and their metabolites in blood and urine. Multicomponent exposures, like in the case of metal-working lubricants, also give rise to analytical problems in the field of biological monitoring. This is also true for some other groups of substances like polycyclic aromatic hydrocarbons (PAH) or nitrosoamines. Concerning the analysis of PAH, however, there seems to be some progress. In our laboratory, a HPLC method is used which allows the simultaneous quantification of 1-hydroxypyrene, 1-, 2+9-, 3-, and 4-hydroxyphenanthrene in urine samples of persons environmentally and occupationally exposed to PAH [12]. Another problem in the analysis of hazardous substances in biological materials is the lack of suitable control and reference materials and external quality assessment schemes.

The search for effect parameters had much less success than that for indices for internal exposure. In the last 15 years there has been little progress in the assessment of biological effect parameters like δ-aminolevulinic acid. On the contrary, great progress has been made in the field of biochemical effect monitoring since Ehrenberg's first publication on this subject in 1977 [13]. There has been considerable research interest in the potential for using the so-called adducts of both DNA and proteins as indicators for potential carcinogenic risk. The binding to DNA as the target molecule is postulated to be the initial step in carcinogenesis. However, the amounts of DNA that can be obtained under monitoring conditions are too small to allow a specific assessment of the adduct level.

In the case of aromatic amines, Neumann [14] showed in 1984 that the level of tissue DNA adducts correlated to the level of haemoglobin adducts (Hb-adducts). This protein is readily available in sufficient amounts for measurements. The determination of these adducts represents a great progress in the prevention of occupational cancer for two reasons:

a) DNA and Hb-adducts can complete the results of biological assays commonly used to predict genotoxic potential;

b) the monitoring of adducts can be used for the assessment of internal exposure and health risks of employees exposed to carcinogenic substances. Thus the adducts provide a better possibility for the measurement of risk assessment than merely detection of substances or their metabolites in body fluids.

Table 4 reports the adducts which are used today for monitoring purposes at the workplace, with the exception of adducts determined by post-labelling methods, because they are not specific for the absorbed substance [15]. For epoxides of alkenes like ethylene, propylene and styrene, adducts can be determined after an Edman degradation as conjugates to N-terminal valine [16]. Determination of the adducts of aromatic amines or PAH-metabolites is simpler to some extent, as, after hydrolysis, these substances and their metabolites can be determined without further treatment.

In spite of important progress in the field of adduct monitoring, it has to be pointed out that today this monitoring is only available for very few substances.

A further limitation for the application of biological monitoring in occupational health is the fact that at present there is only a very limited number of limit values like BAT- or BEI-values. The DFG has so far evaluated 55 BAT-values and the American Conference of Governmental Industrial Hygienists (ACGIH) has published 65 BEI-values, which, unfortunately, cover the same substances in most cases.

Table 3. - BAT-values for organic solvents as evaluated in Germany

Solvent	Parameter	Biological fluid	BAT-value
Acetone	acetone	urine	40 mg/l
2-Bromo-2-chloro-1,1,1,-trifluoroethane	trifluoroacetic acid	blood	2.5 mg/l
2-Butanone (methylethylketone)	2-butanone	urine	5 mg/l
Chlorobenzene	4-chlorocatechols	urine	300 mg/g creatinine
Dichloromethane	CO-Hb dichloromethane	blood blood	5% 1 mg/l
Dimethylformamide	N-methylformamide	urine	15 mg/l
2-Ethoxyethanol	ethoxyacetic acid	urine	50 mg/l
2-Ethoxyethylacetate	ethoxyacetic acid	urine	50 mg/l
Hexachlorobenzene	hexachlorobenzene	plasma/serum	150 µg/l
n-hexane	2,5-hexanedion plus 4,5-dihydroxy-2-hexanone	urine	5 mg/l
2-hexanone	2,5-hexanedione plus 4,5-dihydroxy 2-hexanone	urine	5 mg/l
Carbondisulfide	2-thioxo-thiazolidine-4 carboxylic acid (TTCA)	urine	8 mg/l
Methanol	methanol	urine	30 mg/l
4-Methylpentane-2-one	4-methylpentane-2-one	urine	3.5 mg/l
2-Propanol	acetone acetone	blood urine	50 mg/l 50 mg/l
Styrene	mandelic acid mandelic acid plus phenylglyoxylic acid	urine urine	2000 mg/l 2500 mg/l
Tetrachloroethene	tetrachloroethene	blood	1 mg/l
Tetrachloromethane	tetrachloromethane	blood	70 µg/l
Tetrahydrofurane	tetrahydrofurane	urine	8 mg/l
Toluene	toluene	blood	1.7 mg/l
1,1,1-Trichloroethene	trichloroethanol trichloroacetic acid	blood urine	5 mg/l 100 mg/l
Xylene (all isomers)	xylene methylhippuric acid	blood urine	1.5 mg/l 2000 mg/l

Keeping in mind that about 1000 substances are of really great occupational concern, the number of existing limit values seems to be negligible. The situation appears better, however, if one bears in mind that DFG provides about 170 and NIOSH about 50 analytical procedures for the determination of chemicals in body fluids. That means that biological monitoring is possible for many cases where limit values could have not yet been established. In these cases, biological monitoring is not only possible, but necessary because biological monitoring is the prerequisite for the establishment of limit values. Only in this way internal exposure and health effects can be correlated.

The limitations of biological monitoring can be summarised as follows:

a) for some groups of substances there is a lack of analytical methods and of control and reference values;

b) despite considerable research in the field of biological and biochemical effect monitoring, large gaps have still to be filled;

c) the number of limit values evaluated up to now is very small.

There is but one limitation for biological monitoring which is beyond the possibilities of the scientist, that is politics in the widest sense. Politics seems to be the most serious limitation for biological monitoring.

Table 4. - Adducts which are today used for monitoring purpose at the workplaces

Substance	Target/Analyte	Method	References
Ethylene/ethylene oxide	Hb/hydroxyethylvaline	GC/MS - NCI	[18]
Propylene/propylene oxide	Hb/hydroxypropylvaline	GC/MS - NCI	[19]
Styrene/styrene oxide	Hb/hydroxyphenylethylvaline	GC/MS - NCI	[20]
Aniline	Hb/aniline	GC/NPD	[21]
4-Aminobiphenyl	Hb/4-aminobiphenyl	GC/MS - NCI	[22]
Benzo[a]pyrene (B [a])	Hb/B [a]-tetrols	HPLC/SFS	[23]
	Hb/B [a]-tetrols	GC/MS - NCI	[24]
	DNA/B [a]-DNA-adducts	ELISA	[25]

NCI: negative chemical ionisation; NPD: nitrogen phosphorus detector; SFS: synchronous fluorescence spectroscopy.

The application of biological monitoring is regulated in the various countries in Europe in completely different ways. While biological monitoring is required by law in Germany, other countries only carry out biological monitoring where there are corresponding instructions from the European Community. This only applies, however, for very few substances, e.g. lead.

As the Common European Market is at a stage nearing completion, the European Union (EU) and its activities are of great importance. No member country will be able to get past the decisions of the EU for any length of time.

All documents published since 1984 by the European Commission include the basic principles of prevention: ambient monitoring, biological monitoring, limit values and health surveillance. In the mid 80's there was a decisive change. In 1985 a new concept was agreed upon for the completion of the common market. Article 118 of the Common European Act states that in future in Europe there is to be a harmonisation of health protection on the level of minimum regulations. This had the effect that the subsequent guidelines from the EU no longer contain biological monitoring. This represents an unfavourable sign for the development of biological monitoring in the EU.

However, there seems to be a change in this development, because, meanwhile, an amended proposal for a "Council Directive on the protection of the health and safety of workers from the risk related to chemical agents at work" is under discussion [17]. This proposal states that biological monitoring should be part of health surveillance. Nevertheless, only seven metals (As and its compounds, Be, Cd and its compounds, chromates (Cr^{6+}), Co, Pb and its compounds, Hg and its compounds) and three organic compounds (carbon disulfide, organo-phosphoric esters, tetrachloroethane) are mentioned in this proposal.

Regardless of what is politically required in this area, the major point is that for the individual worker there is no more effective protection against the effects of hazardous substances at the workplace than biological monitoring.

Submitted on invitation.
Accepted on 1 February 1996.

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