Toxicology Letters 192 (2010) 3–16

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Introduction

Biomonitoring for occupational health risk assessment (BOHRA)☆

ARTICLE INFO

Keywords:
Biological monitoring
Occupational health
Risk assessment

ABSTRACT

Biological monitoring (BM or biomonitoring) deals with the assessment of individual human exposure, effect and susceptibility to occupational risk factors. It is a fundamental tool in occupational health risk assessment (OHRA) and occupational health practice (OHP) and it has become one of the most, if not the most active area in occupational health (OH) research today. From the few hundred BM papers published in the 80s, there are now several tens of thousand papers published in the peer review literature each year, and the trend is still rising exponentially. As a result, BM has become a priority for the Scientific Committee on Occupational Toxicology (SCOT) of the International Commission on Occupational Health (ICOH). Moreover, there has been a long-term interest in biological monitoring by other SCs of ICOH such as the Scientific Committees on Toxicology of Metals (SCTM) and on Rural Health (SCRH).

Despite its current popularity, though, BM is not always correctly used or interpreted by those involved in OHRA or OHP. The present review has been prepared to fill this gap and to help preventing misuse and misinterpretation of data. Although the document is meant to be a reference primarily for those involved in OH research and/or practice, it might become of interest for a wider audience within and outside ICOH, including scientists, occupational physicians, industrial hygienists and occupational or public health professionals in general, involved in chemical risk assessment for occupational health. The mission of SCOT and also of other SCs of ICOH, such as SCTM and SCRH, is indeed to promote the advancement and diffusion of knowledge on biological monitoring and other relevant occupational toxicology aspects and to make them available and useful to the entire OH scientific community.

All articles retrieved as of 3 January, 2007 as "Review" with the combined key words "biological monitoring" in PubMed from 2000 to 2007 have been scanned individually. This yielded a total of 1400 articles from a grand total of 2486 (excluding limitation on year of publication). When the title was related to human occupational biological monitoring, the abstract was read and its content was included. Articles outside the 2000–2007 time frame or that are not classified as "Review" in PubMed have also been included, when relevant.

The review is in four parts: (a) the introduction, containing the basic principles and definitions of BM and the different types of biomarkers (BMK), their toxicological significance, practical use and limitations, (b) the methodological and analytical aspects of BM in exposed workers, (c) the interpretation and management of BM data, including a number of recommendations to be considered when planning, performing and interpreting BM results and, finally, (d) the ethical aspects of BM. A list of key references to relevant papers or documents has been included. The BM of specific chemicals or groups of chemicals is outside the purpose of the review.

The document is aimed to represent the state of the art on biological monitoring in occupational risk assessment. We expect that reference to its content will be made, whenever appropriate, by those involved in occupational health practice and research when dealing with BM issues. The document is not meant, though, to represent a rigid nor a permanent set of rules and it will be periodically updated according to new developments and any significant advance in BM science. Any part of the document, therefore, is open to suggestions by scientifically qualified persons or institutions officially involved in BM and comments should be sent directly to the authors. A preliminary draft of the document has been presented at the 7th International Symposium on Biological Monitoring, Beijing, 10–12 September, 2007.

Notes on the literature survey for the preparation of this review

All articles retrieved as of January 3, 2007 as "Review" with the key words "biological monitoring" (no operator, just the two words side by side) in PubMed from 2000 to 2007 have been scanned individually. This yielded a total of 1400 articles from a grand total of 2486 (excluding limitation on year of publication).

When the title was related to human occupational biological monitoring, the abstract was read and, when appropriate for the purpose of the current review, the article was retrieved in EndNote. Four key documents are also cited: the National Academy of Sciences

☆ Prepared by ICOH-*SCOT* in collaboration with ICOH-*SCTM* and ICOH-*SCRH* as part of the Proceedings of ISBM-07, Beijing, 10–12 September 2007.

0378-4274/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2009.05.001

report on biomonitoring (Committee on Human Biomonitoring for Environmental Toxicants, 2006), the WHO document on validation of biomarkers (WHO, 2001), the ACGIH Introduction to BEI®s (ACGIH, 2005) and its German counterpart on BATs (Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, 2005).

Contributors of this document also added references from their own knowledge of the field and own bibliographic search. These references have extended sometimes outside the 2000–2007 time frame retained here as a starting point. They have also included articles that are not classified as "Review" in PubMed. Finally, further discussion within the scientific community should occur on the proposed outline (e.g. introduction, methods in BM, interpretation and management of BM data, ethical considerations) before this review is submitted to ICOH for approval. The current review is the authors' responsibility and it is only meant to be a starting point or a teaser for the proposed consensus document to be possibly approved officially by ICOH.

1. Introduction

A number of extensive and detailed high quality review papers have already been published on various aspects of human biological monitoring (BM or biomonitoring). These publications have been the result of the collaborative effort of several groups of experts, and provide an invaluable contribution to harmonize the scientific approach to BM and facilitate the practical work of those involved in BM in different areas of the world. Within ICOH the SCTM has long recognized the importance of BM and published several consensus documents related to BM, e.g. TGMA (1973), Clarkson et al. (1988) where risk assessment is specifically addressed, and, recently, the 3rd edition of the Handbook on the Toxicology of Metals (Nordberg et al., 2007) where risk assessment is specifically addressed (Nordberg and Fowler, 2007). These publications reflect the background of the authors, the scientific context in which the reviews were prepared and, of course, their specific purpose. The Environmental Health Criteria 222 on Biomarkers in Risk Assessment: Validity and Validation (WHO, 2001), for example, is focused on methodological issues, whereas the more recent publication Human Biomonitoring for Environmental Chemicals prepared by the Committee on Human Biomonitoring for Environmental Toxicants (NRC-USA, 2006) is focused on the role of biomonitoring as an exposure assessment tool finalized to public health efforts. In the widely known Introduction to Biological Exposure Indices (BEI®), a classic reference text for industrial hygienists and professional BM operators updated yearly by the ACGIH, discussion is focused on exposure biomarkers (ACGIH, 2005). Moreover, a number of publications have addressed important aspects of BM, including terminology, related to specific risk factors such as toxic metals and pesticides (IUPAC, 2003, 2006, 2007).

The Scientific Committee on Occupational Toxicology (SCOT) has noticed that none of these efforts has specifically addressed, in a comprehensive and exhaustive way, all the general issues related to the design, use and interpretation of biomonitoring in occupational health (OH). Considering the increasing role, in recent years, of BM in occupational health practice and, also, the difficulties and limitations still existing in the correct use and interpretation of different biomarkers, particularly of newly developed and validated biomarkers, SCOT has considered it was the time to take the challenge of publishing a document which may represent the consensus currently existing on BM within the OH community. The difficulty has been, therefore, to extract from the exploding BM arena those concepts which are on the one hand most relevant for their use by OH professionals (OHPs) and on the other a common basis for improving harmonization and quality in BM protocols.

The focus of this document is to address, in a concise and synthetic form, the most relevant areas of interest for the OHP, i.e. planning, implementing, interpreting and communicating the results of BM studies and protocols. Attention has been made to highlight advantages and limitations of BM versus other tools used for risk assessment such as environmental monitoring, health surveillance, animal experimentation and modelling. A clear distinction has to be made between biomarkers for use in research, i.e. those tests which have not been yet fully validated for a routine application, and biomarkers commonly used in OH practice, i.e. those tests which have already been interpreted and validated in the scientific literature and can, therefore, be routinely used for occupational health purposes. This distinction is particularly important in relation with susceptibility biomarkers, many of which have not yet been sufficiently validated in the workplace.

1.1. Definition and significance of biological monitoring (BM)

A number of similar, although not identical, definitions of biological monitoring exist (Zielhuis and Henderson, 1986). For the purpose of the present document BM is defined as the repeated, controlled measurement of chemical or biochemical markers in fluids, tissues or other accessible samples from subjects exposed or exposed in the past or to be exposed to chemical, physical or biological risk factors in the workplace and/or the general environment. This document will focus on biomonitoring for chemical risks. BM of workers has three main aims: the primary is individual or collective exposure assessment, the second is health protection and the ultimate objective is occupational health risk assessment. BM consists of standardized protocols aiming to the periodic detection of early, preferably reversible, biological signs which are indicative, if compared with adequate reference values, of an actual or potential condition of exposure, effect or susceptibility possibly resulting in health damage or disease. These signs are referred to as biomarkers. The periodicity of measurement is important to ensure that any early change is timely detected. The validity (sensitivity and specificity) of a biomarker is, however, the single most important aspect to be considered. Sensitivity, i.e. the ability to avoid false negative results, is fundamental for preventive purposes, whereas specificity, i.e. the capacity to avoid false positive results, is usually more important for diagnostic purposes.

1.2. Role of BM in exposure assessment

The term BM has come into use as a natural adaptation of the term environmental monitoring (EM), i.e. the periodic measurement of the level or concentration of a chemical, physical or biological risk factor in the workplace environment, which is traditionally used as an indirect measure of human exposure. Indeed, the most frequent use of biological monitoring is for assessing individual exposure to chemicals by different routes (inhalation, dermal and ingestion). Measurements of the concentration of substances or their metabolites in urine, for example, can provide useful information to assess inadvertent ingestion, but only in conjunction with measurements of exposure by other relevant routes such as inhalation and/or dermal (Cherrie et al., 2006). On the other hand, biomarkers of exposure should be used with care when single routes of absorption have to be assessed. For example, while biomonitoring can provide valuable information on dermal uptake in controlled conditions, it must be used with care in assessing the amount of dermal exposure in workplaces where the chemical may be additionally absorbed by inhalation or ingestion (Semple, 2004).

When compared to EM, BM provides additional information which can be effective in improving occupational risk assessment at the individual and/or group level. This information includes the assessment of the integrated total uptake of the chemical by dif-

ferent routes of absorption, an estimate of current, recent or past exposure, the degree of metabolism, the early biological effects and the ability of an organism to tolerate and respond to chemical insult. Without BM as a tool none of the features above, and particularly the contribution of all routes to exposure, including dermal exposure, nor the role of variability factors in absorption, metabolism and excretion, could be investigated, and occupational risk assessment of chemicals would be more uncertain and vague than it is.

In environmental epidemiological studies, biological measures of exposure should be preferred, if available, to environmental exposure data, as they are closer to the target organ dose and provide greater precision in risk estimates and in dose–response relationships. Suitable measures for epidemiologists to use in population studies are now becoming increasingly available (Sim, 2002). This is the case, for example, when biomonitoring exposure to complex mixtures, such as PAHs from polluted ambient air, diesel exhaust, tobacco smoke or the above together. This is per se a particularly challenging exercise since such types of exposures have many constituents in common and people are usually exposed to more than one of these mixtures at the same time (Scherer, 2005). However, biological monitoring and environmental monitoring are not alternative but rather complementary tools.

1.3. Role of BM in occupational health

Another important application of BM, besides exposure assessment, is the use of biomarkers, at either individual or group level, for the correct interpretation of doubtful clinical tests. These are usually performed as part of an occupational health surveillance program when EM data are unavailable or are deemed unreliable. Health surveillance is the periodical assessment of the workers' health status by clinical, biochemical, imaging or instrumental testing to detect any clinically relevant, occupation-dependent change of the single worker's health. Biomarkers are not only useful for assessing internal exposure or, in the absence of EM data, even external exposure, or for predicting potential health effects in the absence of clinical impairments. They are usually also more specific and sensitive than most clinical tests and may be more effective, therefore, for assessing a causal relationship between health impairment and chemical exposure when a change is first detected in exposed workers. This kind of assessment requires, however, that the dose–response relationship be known between the level of biomarker actually measured and the level of risk, i.e. the prevalence or incidence of a specific adverse effect in a representative exposed populations. Two examples of the practical utilization of BM to interpret positive clinical findings, i.e. for the assessment of individual job fitness, are provided here.

The first example relates to the concept of "exposed workers". In order to classify a group of workers as "exposed" starting from BM data, a comparison is necessary between the levels of a given exposure biomarker measured in those workers and those measured in a corresponding, non-exposed (but otherwise identical) population to be used as a control group. These levels are known as "reference values". Only if the level(s) found in the worker, or group of workers, are significantly higher than the reference values, may the worker(s) be considered truly "exposed". This does not mean, however, that any chemical-related impairment observed in that worker(s), although still compatible with the specific toxicological profile of the chemical involved (dose–effect), can be attributed to occupational exposure. For this attribute to be considered as correct, it is necessary that the level of exposure measured in the worker(s) corresponds to that known from the epidemiologic literature to be qualitatively and quantitatively associated with that specific health effect (dose–response).

The other relevant example of the need for a sound case by case health risk assessment comes from the comparison of the level of a biomarker found in an individual worker with the corresponding Biological Exposure Index (BEI®), i.e. the average level found in groups of workers exposed to the threshold limit value (TLV®, the limit concentration recommended by the ACGIH for airborne occupational exposure). If an individual showing a risk-compatible health impairment has an increased or even a very high level of a biomarker of exposure as compared with the BEI® value, this, per se, does not necessarily mean that the health problem is actually due to occupational exposure (false positive). In fact, the opposite is also true: the observation of a level of a biomarker which is lower than the BEI® in an exposed subject does not per se exclude a possible role of occupational exposure in the health impairment observed (false negative). In the latter case it is possible that the worker is hyper-susceptible and that the biomarker per se underestimates his/her individual risk.

These examples suggest caution in the interpretation of BM results and show the intrinsic limitations of BM when used for individual health risk assessment. They underline the ultimate role of the OHP for a correct interpretation of BM data, particularly when used for either clinical or legal purposes (Manno and Cocheo, 1989).

An additional, increasingly recognized, advantage of BM versus EM is the possibility to quantify the long-term interaction of the organism with persistent environmental chemicals (i.e. toxic metals, halogenated hydrocarbons and pesticides) and the resulting potentially harmful impact on living organisms, including but not limited to human beings. The use of biomarkers to assess chemical persistence can provide a measure of the past exposure, the early adverse effect and the individual susceptibility to these persistent environmental chemicals and may be particularly useful to assess and control the risk of long-term outcomes associated with their exposure (Gil and Pla, 2001). This aspect, although important, is not directly related to occupational risks and, therefore, will not be addressed in this document.

1.4. BM and occupational risk assessment (ORA)

BM has a fundamental role in, but it is not limited to occupational risk assessment. Experience in BM gained in the occupational setting has often been applied to assess (the effects of) human exposure to chemicals in the general environment. The use of biological fluids/tissues for the assessment of human exposure, effect or susceptibility to chemicals in the workplace represents, together with the underlying data (e.g. personal exposure and biological monitoring measurements, media-specific residue measurements, product use and time-activity information), a critical component of the occupational risk assessment process, a rapidly advancing science (Ross et al., 2006). The definition and principles of risk assessment will be reviewed briefly and specific applications of BM for occupational risk assessment will be presented.

1.4.1. Definition and principles of ORA

Occupational risk assessment (ORA) may be defined as the qualitative and quantitative characterization of an occupational risk, i.e. the probability that an adverse health effect may result from human exposure to an occupational toxic agent. ORA has three fundamental tools: environmental monitoring, health surveillance (HS) and biological monitoring.

For the purpose of this document, risk assessment is meant to quantify the likelihood that a quantitatively defined occupational exposure of an individual (or group of individuals) to a given chemical might result in some adverse health effects. The level of probability essentially depends on three elements: the intrinsic potency/characteristics of the risk factor itself (hazard), the level/type/duration of exposure and the degree of individual sus-

ceptibility, as represented by the following simple equation:

$$\text{Risk} = \text{Hazard} \times \text{Exposure} \times \text{Susceptibility}$$

The equation states that for an appreciable level of risk to be present, each of the three components must be different from zero. It also indicates that the same level of risk may be achieved by various associations of different levels of each of the three components. Highly intrinsically toxic chemicals, including carcinogens, at low levels of exposure and/or susceptibility may provide the same or a similar level of risk as would less toxic ones do at higher levels of exposure and/or susceptibility. This means that hypersusceptibility becomes increasingly important at lower levels of exposure, whereas it has little or no importance at high exposure. These concepts have important practical implications and should be born in mind by the occupational physician or hygienist in planning a BM program, when, for example, due to cost, time or other types of limitation, he/she has to choose which test, among the different exposure, effect or susceptibility biomarkers possibly available, will be more informative, effective and efficient in terms of ORA.

The classic, general definition of risk assessment by the National Research Council (NRC, 1983) is based on the following four steps, with BM contributing to each of them, although to a different extent:

(a) *Hazard identification*: The observation of an increased individual or group level of a potentially toxic chemical, or its metabolite(s), in blood, urine or other biological samples from exposed workers as compared to those of control individuals. BM may provide strong indirect evidence for the presence of the chemical in the workplace.
(b) *Definition of the dose–response relationship*: BM can contribute to estimate either side of the equation, i.e. it may help to measure the dose (as the biological level of a chemical or its metabolite(s) corresponding to a given level of exposure) or to detect the response (the proportion of individuals showing some early adverse effect at a given level of exposure) or both.
(c) *Exposure assessment*: This is probably the most common situation for the use of BM data, at the individual or group level, to the purpose of ORA. BM would be expected to show a correlation between the levels of biomarker(s) and the levels of exposure of the workers, individually or as a group, to the chemical in the workplace. BM is often more specific and sensitive than EM in assessing the degree of recent and, by all means, also past exposure to chemicals from all routes.
(d) *Risk characterization*: Finally, BM can also be used to perform or validate ORA when the other approaches, i.e. EM and HS, are unavailable or inadequate due to an intrinsically low sensitivity and/or specificity. BM also allows one to assess specific, otherwise inaccessible, components of risk, such as metabolic polymorphism, enzymatic inhibition or induction of the metabolizing enzymes and other susceptibility factors which may be responsible for a different response to chemicals.

1.4.2. Contribution of BM to ORA

Due to intra-individual and inter-individual biomarker variability, risk assessment based on BM data is usually best applied to the workers as a group (e.g. levels of biomarkers of exposure at the beginning vs. the end of the work shift or levels measured in exposed vs. non-exposed or less exposed subjects). In some cases, though, information may be used directly to the individual worker's benefit (e.g. biomarkers of past exposure, early effect or susceptibility).

Some of the most common benefits to ORA from the use of BM data are the following:

- the assessment of the total internal dose from all different routes of absorption (including inhalation, ingestion, dermal absorption) or from a single route of absorption when the others are excluded;
- quantitation of the internal dose from exposure to multiple chemicals, including assessment of interaction/competition in absorption, metabolism, excretion;
- separation between occupational and non-occupational exposure (e.g. pre-shift vs. post-shift values for volatile or, in general, short half-life chemicals);
- estimate of past exposure (e.g. PbU determination after Pb chelation by EDTA);
- assessment of protective equipment efficacy, ventilation, workplace amelioration, etc.;
- assessment of individual susceptibility (e.g. genetic polymorphism, metabolic phenotype, DNA repair, etc.);
- assessment of early signs of disease (i.e. late biomarkers of effect).

1.5. Advantages and limitations of BM

The advantages of using biomarkers as tools for exposure assessment are well established. Biomarkers are particularly useful when their toxicological significance is sufficiently understood, including the following: the toxicokinetic fate of the chemical or its metabolites (for exposure biomarkers), or the mechanism of the disease/adverse effect (for effect biomarkers), or the modulating factors linking the chemical to the disease/adverse effect (for susceptibility biomarkers). Biomarkers are less useful if their toxicological significance is unknown or unclear. This is sometimes the case for pesticides: since they are necessarily toxic, because they aim at controlling undesired living species, and they are voluntarily spread into the environment to reach their targets, their use always poses some risks to human health that need to be evaluated. A better knowledge of pesticide metabolism in humans and the setting of specific biological exposure indices (BEIs) would make the use of biological monitoring for the risk assessment of human exposure to pesticides more effective (Maroni et al., 2000). This is becoming increasingly important nowadays, due to the introduction in the market of an increasing number of new compounds, for which no biomarkers are available.

Sometimes it is not easy to clearly distinguish between different types of biomarkers, i.e. biomarkers of exposure, effect or susceptibility. This is the case, for example, of biomarkers used for the assessment of organophosphate (OP) exposure such as serum pseudo-cholinesterase activity inhibition, an effect biomarker per se, but without any toxicological significance since pseudocholinesterase and acetylcholinesterase, the critical target of OP toxicity, are totally unrelated enzymes. Another case is represented by a number of potential biomarkers of susceptibility such as those related with different toxicologically relevant phenotypes of drug metabolizing enzymes such as cytochrome P450 (CYP). When measuring, for example, CYP2E1 enzymatic activity in vivo, using a specific substrate as a biomarker of metabolic susceptibility in subjects exposed to chlorinated solvents or other organic chemicals, a decrease (or increase) in the enzymatic activity may in fact indicate that exposure to chemicals which are CYP2E1 substrates has occurred (Lucas et al., 1999). The type and degree of the metabolic effect to be expected may be very different, depending on whether competitive or non-competitive inhibition or induction of the enzyme activity has actually occurred.

There are huge differences throughout the world in the use of BM in occupational health, not only for medical or economical reasons but also for the existence of different cultural, ethical and social contexts. For instance, important discrepancies have been noticed between Europe and the United States regarding the role and use of biological monitoring for occupational exposure/health assessment. Through the use of biomarkers of effect BM has become

an important tool for medical health surveillance in many European countries. In the United States, instead, and in the UK, where it is mainly used for exposure assessment by both occupational physicians and industrial hygienists, BM belongs rather more to the field of occupational hygiene than to occupational health. BM has become also increasingly important in the health risk assessment of chemicals from non-occupational environmental exposure. In Asia it is increasingly used in toxicology and in occupational and environmental health practice, even in industrially developing countries. In China, for example, 17 occupational exposure limits have been established. For all these reasons it has been suggested that it would be worthwhile to include BM in the curricula for the training of occupational hygienists as it is already included in that of occupational physicians (Jakubowski and Trzcinka-Ochocka, 2005).

Other important differences in the use of BM in occupational health are observed among different productive activities. BM is somehow easier to use in those cases or industries, where levels of exposure are relatively stable over time (indoor environment, continuous production cycle) and therefore the repeatability and representativity of the collected biological measures are better. On the other hand, reliable data are more difficult to obtain in agriculture, where a great number of factors cause significant variation of the levels of exposure of the workers, thus affecting the reliability of the measurements more strongly (climatic conditions, intermittent use of agrochemicals, use of mixtures whose components significantly vary over time, etc.). This is the reason why algorithms and models are preferably being developed in agriculture, where they can be used, with any biological and environmental data available, in the frame of an integrated approach to risk assessment.

1.6. Classification of biomarkers

Biomarkers have been defined by the National Academy of Sciences (USA) as an alteration in cellular or biochemical components, processes, structure or functions that is measurable in a biological system or sample. BM requires defined procedures for planning, appropriate protocols for implementation, correct sampling and data collection, and rigorous criteria for interpreting the results obtained.

The traditional, generally accepted classification of biomarkers into three main categories (i.e. biomarkers of exposure, effect, and susceptibility) depending on their toxicological significance (WHO, 2001; IUPAC, 2004, 2006, 2007) is still followed here. A biomarker of exposure is a chemical or its metabolite or the product of an interaction between a chemical and some target molecule or macromolecule that is measured in a compartment or a fluid of an organism. A biomarker of effect is a measurable biochemical, structural, functional, behavioural or any other kind of alteration in an organism that, according to its magnitude, can be associated with an established or potential health impairment or disease. A sub-class of biomarkers of effect is represented by biomarkers of early disease (or early biomarkers of disease), i.e. tests which are more closely indicative of a subclinical effect or even an early, reversible clinical response. Finally, a biomarker of susceptibility may be defined as an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a chemical.

Although the different types of biomarkers are considered, for classification purposes, as separate and alternative, in fact it is not always possible to attribute them to a single category. The allocation of a biomarker to one type or the other sometimes depends on its toxicological significance and the specific context in which the test is being used. The measurement of DNA– or protein–chemical adducts, for instance, may be interpreted differently, i.e. as an indicator of either internal exposure or biologically effective dose or early effect or even susceptibility, depending on the time of sampling, the target organ for toxicity, the type of effect on DNA or protein, the role of the gene(s) or enzyme(s) involved, etc. Benzene–DNA adducts in human lymphocytes provide a useful example: not only are they biomarkers of exposure, but possibly also biomarkers of effect (being benzene a genotoxic carcinogen) and biomarkers of susceptibility (indicating subjects with high CYP2E1-dependent benzene bioactivation).

1.6.1. Biomarkers of exposure

The fundamental role of biomarkers of exposure in OH practice is to assess exposure by all routes and to complement information obtained by workplace environmental monitoring. Environmental exposure data, even through personal sampling, may considerably underestimate or overestimate the actual internal dose (bioavailability) of a chemical, or its metabolites, in an individual. BM data are usually more informative, particularly at the individual level, than those from environmental monitoring and most of the information provided by BM simply could not be obtained by environmental monitoring procedures alone. This is the case, for example, for chemicals with significant absorption by dermal exposure or ingestion or for chemicals with a wide inter-individual variability in absorption, metabolism, excretion. BM can also be indicative of the individual workload, of multiple exposures, of recent versus past exposures, etc. In this respect kinetics are important in the interpretation of biomarkers' results. Different chemicals in the same medium may have different significance depending on their half-life which varies dramatically, for example, from months to years for mercury or cadmium in urine, or minutes to years for benzene or PCBs in blood, respectively.

For all these reasons biomarkers of exposure are often used, when available, as a better substitute for EM. Due to the large inter-individual variability in absorption, distribution, metabolism and excretion of chemicals, or their metabolites, individual BM data may vary extensively as compared with the corresponding environmental exposure data, thus providing a more accurate assessment of individual exposure. A limitation though in the use of BM is its inability to discriminate between different sources of exposure. In some cases, exposure from the general environment may contribute significantly to occupational exposure and complicate the understanding of the relative contribution of the two routes to the total dose absorbed. This is why in most instances the integrated use of both environmental and biological measurements is still the best approach to individual exposure assessment.

When a chemical has a set of different available biomarkers, their concurrent measurement will provide a much better estimate of the degree, time and conditions of exposure and a better estimate of risk than measuring a single biomarker alone. A classical example is to perform risk assessment in subjects occupationally exposed to Pb through the periodical measurement of both exposure and effect biomarkers. The measurement of Pb and protoporphyrin IX levels in blood together with that of Pb (with and without chelation by EDTA), D-ALA and porphyrins in urine would provide a protocol for a fairly accurate and exhaustive individual risk assessment in these subjects.

When the dose–response relationship is known between the level of exposure, or the corresponding biomarker of exposure, and the probability of a given adverse biological effect, the biomarker can provide also a reasonably accurate estimate of risk at the group level and, sometimes, even at the individual level. When such a relationship is not known, the biomarker of exposure will only indicate the dose actually absorbed and, at best, the cumulative level of exposure by all routes. In either case the enormous recent progress in analytical chemistry and biochemistry is providing more and more sensitive and specific methods for risk/exposure assessment.

A special group of exposure biomarkers is represented by protein and DNA adducts, i.e. the products of the interaction between a reactive chemical or a metabolite and a target molecule. The so-

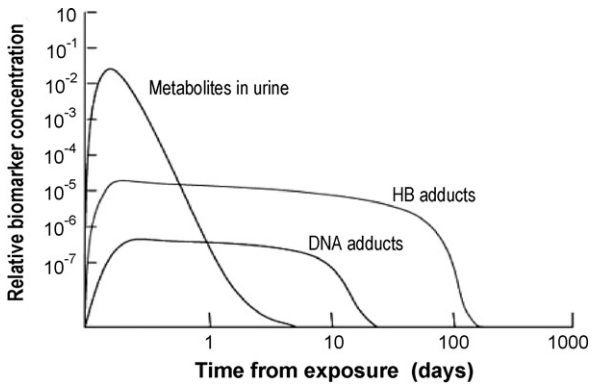

Fig. 1. Lifespan of different biomarkers of exposure (Henderson et al., 1989).

called "biologically effective dose" or, more simply, the "target dose" represents the small amount of a chemical absorbed by the organism that has reacted with a specific target molecule, usually DNA or protein (Hb). Although such a measurement remains a surrogate for something impossible to measure (i.e. the total amount of the chemical undergoing activation), it has several advantages over more traditional exposure indicators. One advantage is that one can estimate the proportion of the chemical which has undergone bioactivation. Another advantage is the possibility to estimate the time at which exposure has occurred, according to the half-life of the target molecule (Fig. 1). Finally, based on their toxicological significance, they are considered as "multifunctional biomarkers": i.e. biomarkers of exposure since their level depends on the level of exposure, of effect since they indicate a damage to the target molecule, and of susceptibility since they reflect the degree of metabolic activation and/or DNA repair.

Hb adduct determination, in particular, has three major advantages as compared to DNA adduct measurement: (1) it provides qualitative information on the structure of the reactive intermediates, which may be obtained through mass spectrometry and may provide insight on the molecular mechanism of toxicity of compounds such as butadiene (BD) or benzene; (2) it allows the possibility of reliable determination of exposure over periods of several months with a limited number of samples, e.g. for compounds such as ethylene oxide (EO), propylene oxide (PO) and BD which form stable adducts with Hb (Boogaard, 2002); and finally (3) it is long-lasting since there is no known repair mechanism for adducts to proteins.

DNA adducts, on the other hand, are promising markers of carcinogen exposure, providing an integrated measurement of carcinogen intake (exposure), metabolic activation (susceptibility), and delivery to/modification of a critical macromolecule in target tissues (effect). Detecting the presence of DNA adducts in human tissues, although per se not a marker of cancer, is a particularly useful tool for molecular epidemiological studies of cancer. Moreover, monitoring accessible surrogate tissues, such as white blood cells in subjects exposed to benzene, provides, for example, a means of investigating occupational or environmental exposure in healthy individuals which is better risk-related than measuring benzene levels in air or its metabolites in urine (Phillips, 2005).

1.6.2. Biomarkers of effect

This type of biomarker indicates early biochemical or functional alterations including a wide array of biological responses, ranging from physiological adaptation to disease. They represent a heterogeneous group of indicators and have different applications depending on the toxicological significance. Some of them have been used for decades as indirect biological signs of exposure rather than markers of effect. This is because they are well and promptly correlated with the degree of exposure, sometimes, but not always, even at levels of exposure without any toxicological significance. This is the case, for example, of the measurement of red blood cell acetylcholinesterase (AChE) activity in subjects exposed to compounds acting through acetylcholinesterase inhibition (mainly some organophosphorous and some carbamate compounds). This biomarker still represents a useful tool for ORA, but should be better regarded (and used) according to its true toxicological significance of an effect biomarker.

Other endpoints, such as, for example, proteins in urine of subjects exposed to nephrotoxic solvents or metals, have been largely used as early indicators of biological effect. This application requires, of course, that the target organ and preferably also the mechanism of chemical toxicity be known. In this case the same biomarker(s) can be used as "target organ biomarker(s)" in humans and also to study other chemicals' target organ effects and mechanism of toxicity in experimental animals. However, the opposite is also true: experimental studies in animals may provide or suggest new potential indicators of effect which should then be tested for use as biomarkers of effect in exposed subjects.

A correct use of effect biomarkers in the BM or the health surveillance of exposed workers does not necessarily require that the mechanism of action of the chemical be fully understood. A traditional example of target organ biomarkers is represented by the monitoring of non-specific biochemical molecules or functions in organs such as liver or kidney. Here, however, the distinction between BM and health surveillance becomes slim. Increased levels of aminotransferases or other cytolytic enzymes in serum and/or urine or increased levels of selected proteins in urine are typical examples of non-specific liver and kidney target organ biomarkers. In this case, the degree/persistence of the impairment will classify the test as one of either clinical chemistry or BM, more than its toxicological significance or its physiopathological role in the development of the disease.

An important group of effect biomarkers which have been developed in animals, even in vitro, and are now increasingly applied to occupationally exposed populations, are genotoxicity biomarkers in workers exposed to mutagens or genotoxic carcinogens. These tests, including chromosomal aberrations, micronuclei and the more recent Comet test, may be effective in distinguishing exposed from non-exposed subjects at high exposure. Mainly used as group indicators they are sensitive but not specific and in some cases difficult to interpret correctly, although new techniques, such as the alkaline Comet test, appear to be promising in distinguishing between different mechanisms of DNA damage (covalent binding vs. oxidative stress). This emphasizes the importance of a stronger interaction and collaboration between OHPs or other medical specialists involved in occupational biomonitoring and scientists interested in mechanistic toxicology.

Although biomarkers are usually chemicals, biochemicals or biological functions, a special and controversial group of biomarkers of effect is represented by neurobehavioural tests. These tests, although they do not fit the general definition of biomarkers reported in Section 1.1, are commonly used as sensitive functional markers, alone or together with more traditional biomarkers, in epidemiological studies on workers exposed to solvents or other centrally neurotoxic chemicals. The practical advantages of these tests, as compared with traditional biomarkers such as metabolites in urine, coincide with their limitation: a high sensitivity and a low specificity, thus making the use of neurobehavioural tests for establishing exposure limits controversial. Ideally, validation of biomarkers of response in strictly controlled epidemiological studies would be in fact highly desirable, although ethically sensitive. Transitional epidemiological studies should bridge the gap between research in the laboratory and use in the field. In a transitional study, a potential biomarker of response is the dependent

variable to be evaluated, while another established biomarker or a validated measure of exposure, effect or susceptibility, must be used as the independent variable. Only if/once the biomarker of response is validated can it be used alone to provide valuable data for human health risk assessments or even as a guide for medical surveillance programmes (Albertini, 2001). This is not yet the case for neurobehavioural tests. So, the use of these functional tests for monitoring/studying the effects of chemical exposure in individual workers should be avoided and in groups of workers should be made carefully.

In summary, effect biomarkers used as early predictors of clinical disease can improve occupational health risk assessment and contribute to implement new effective disease prevention policies in occupational and environmental settings, but they must be first validated. The process of validating biomarkers involves dealing with a range of characteristics that include primarily the intrinsic qualities of the biomarker, its determinants and the analytical procedure (Bonassi et al., 2001). Validation also involves the clarification of the biomarker's toxicological significance, which means its relation with the chemical's mechanism of action and its ability to detect or predict a specific toxic effect.

1.6.3. Biomarkers of susceptibility

Hyper-susceptibility can be defined as a lack of capacity, beyond the limits of human variability, to tolerate or respond effectively to exogenous toxicants or pathogens. The concept of individual variability is intrinsic to the interpretation of chemical biomonitoring data as well as to that of any biological or clinical test. Mechanisms of susceptibility to chemical agents are of two kinds: toxicokinetic and toxicodynamic. Biomarkers of susceptibility may be of either type. A group of potential susceptibility biomarkers with a toxicokinetic mechanism for use in humans exposed to chemicals is represented by the in vivo measurement of the specific drug metabolizing enzymes or enzyme activities involved in the chemicals' activation or detoxication reactions. Cytochrome P450 (CYP) is the human enzyme superfamily involved in the bioactivation and/or detoxication of many occupational and environmental chemicals, and the isoform CYP2E1 is specifically mentioned here as an example.

The importance of this isoform derives from its unique substrate spectrum that includes a large number of low molecular size, toxicologically important, high-production chemicals, such as aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, including solvents and industrial monomers, and many others. Possible consequences of differential inter-individual and inter-ethnic susceptibilities may be related to (i) individual expression of clinical signs of chemical toxicity, (ii) biological monitoring data in exposed workers, and (iii) interpretation of results of epidemiological or molecular-epidemiological studies (Bolt et al., 2003).

Polymorphism of other enzymes, such as the glutathione transferases (GST), a group of detoxifying enzymes which catalyze a nucleophilic attack by reduced glutathione on non-polar compounds with an electrophilic carbon, nitrogen or sulphur atom, has also been recognized as an important factor of human susceptibility to chemical toxicity. This is due to the lower detoxication capability associated with allelic variants encoding GST isoforms with a reduced or absent catalytic activity. Another example of enzyme polymorphism being investigated as a possible biomarker of susceptibility is delta-aminolevulinic acid dehydratase (ALAD) genotype and lead toxicity (Kelada et al., 2001).

Despite the intense work ongoing and the promising results achieved on the pharmacological and toxicological significance of polymorphic metabolizing enzymes, their routine use as biomarkers for occupational risk assessment is yet to be validated.

2. Methods of BM

Biomonitoring has been used successfully in many occupational and environmental health or exposure studies for some time now and many analytical methods, sampling strategies, epidemiological data and guidance values are already available. It is becoming clear, however, that we are only now beginning to understand the methodological complexities and uncertainties associated with the biomonitoring process, from study design, to sample collection, to chemical analysis, to the interpretation of the results, individually and collectively (Barr et al., 2006).

2.1. Study design

One of the most critical steps when starting a new BM program is the study design, i.e. the planning of which biomarker(s) should be measured, in which biological fluid or tissue, when and how many samples should be collected and from which workers. In general, non-invasive and easily collected samples are usually preferred. The choice depends also on many other factors such as the toxicokinetics of the chemical(s), including its (their) routes of absorption, metabolism, distribution, accumulation and excretion, the validity (specificity and sensitivity) and toxicological significance of the biomarkers available, their stability and reproducibility, the purpose of the BM program, the size and characteristics of the study population, and many others. An accurate choice of the statistical methods to be used, including their characteristics and limitations must also be made. Finally, an estimate of the cost-benefit and/or risk-benefit analysis, including a detailed evaluation of the ethical procedures and constrains, including information and communication, are always necessary.

2.2. Collection, processing, and storage of samples

Samples for use in BM include fluids (blood, urine, exhaled air, sweat, semen, feces, etc.) and tissues (skin, mucosa, parenchymal tissue, bone, hair, etc.) and depend on the type of biomarker (i.e. of exposure, effect and susceptibility) and/or the type of chemical (parent compound vs. metabolite, volatile vs. non-volatile, hydrophilic vs. hydrophobic, labile vs. persistent, etc.). Sometimes the ratio between the concentration of a metabolite product and that of the parent chemical in blood or urine is the biomarker, not the chemical or the metabolite per se. This is the case, for example, for the genotyping of drug metabolizing enzymes, such as CYP2E1, which has been proposed as a biomarker of susceptibility in humans exposed to benzene or other chemicals which are activated or detoxified by CYP2E1 (Lucas et al., 1999).

Many factors, such as fluid/tissue type, time of collection, containers used, preservatives and other additives, storage temperature, transport means and length of transit time, may affect the quality and stability of the samples and the measurement of biomarkers. All these, therefore, must be considered with care at the initial collection stage. Development of standard operating procedures and quality control plans is a safeguard of the samples' quality and of the validity of the analyses' results (Holland et al., 2003).

Breath analysis is an attractive non-invasive procedure for screening workers exposed to volatile chemicals such as solvents or mercury. It has been used in numerous laboratory based studies and for field research. Despite its obvious advantages, biological monitoring has failed to become widely accepted as a routine tool in occupational hygiene. Recent advances in breath sampling and analysis are such that it is likely to become more widely used in the future (Wilson and Monster, 1999). Exhaled alveolar breath analysis, for example, not only can provide information about an individual's status of health or his exposure to acutely harmful chemicals. It can also be used to quantify recent exposure to

volatile organic compounds, to link particular biomarkers of effect to specific exposures, to determine compound-specific uptake and elimination kinetics, and to assess the relative importance of various routes of exposure (i.e. dermal vs. ingestion vs. inhalation) in multi-pathway scenarios (Lindstrom and Pleil, 2002).

In more recent years hair has become an important biological specimen, alternative to the usual blood and urine samples, for drug testing in the fields of forensic toxicology, clinical toxicology and clinical chemistry. Moreover, hair-testing is now becoming more widely used also in workplace testing, as well as in historical reconstruction and in legal cases (Boumba et al., 2006).

Saliva has been used to evaluate a broad range of biomarkers, drugs, and environmental contaminants, including toxic metals and pesticides. To advance the application of non-invasive biomonitoring, a microfluidic/electrochemical device has also been developed for the analysis of lead (Pb), using square-wave anodic stripping voltammetry (Timchalk et al., 2004).

Biomarker studies allow also processing and storage of numerous biological samples with the goals of obtaining a large amount of information, to be used also in future studies, and minimizing research costs. An efficient study design should ideally include provisions for processing the original samples, such as cryopreservation, DNA isolation, preparation of specimens for future exposure assessment and other potential uses (Holland et al., 2005).

2.3. Analytical methods (e.g. comparison, advantages, disadvantages, precision, sensitivity, specificity and quality assessment)

Recent advances in analytical chemistry have made it possible measuring trace levels of chemicals, their speciation (WHO/IPCS, 2006; Needham et al., 2005) or their metabolites in almost any biological fluid or tissues and have, therefore, contributed significantly to the increased use of biomonitoring in exposure assessment. The application of liquid chromatography–mass spectrometry (LC–MS) in occupational and environmental toxicology, although relatively recent, has been demonstrated as a valid tool in the determination of traditional biomarkers of exposure, as well as in metabolism studies aimed at investigating minor metabolic routes or new, more specific biomarkers (Manini et al., 2004). The most common biomonitoring approach, for example, for investigating human exposure to phthalates is the measurement of urinary concentrations of phthalate metabolites. Biomonitoring data combined with indirect measures of exposure are the most appropriate tools for assessing exposure to phtalates (Hauser and Calafat, 2005). Assessing exposure to phthalates by analyzing urine for their metabolites is preferable to the determination of the parent compounds in air, water and foodstuff because less subject to contamination. Furthermore, these metabolites are involved in phthalates toxicity. Moreover, a frequent biological monitoring of phthalates in body fluids and tissues would also help physicians to perform individual health risk assessments for exposure in the general population and in guiding governments to provide regulations concerning the maximum allowed concentrations in the environment, plasticized products, medications and medical equipment (Latini, 2005).

Even the use of relatively simple biomarkers, such as urine analysis of unchanged solvents in occupational applications, is not yet widespread. Nonetheless, in the short time since its application, a number of important discoveries have been made, and the future appears to be promising for this branch of analysis. The basic concepts and methodology of urine analysis for use in BM have lead a critical revision of the literature on this matter. The excretion mechanisms of organic solvents in urine, with regard to biological and analytical variability, have been reviewed and the future directions of research discussed (Imbriani and Ghittori, 2005).

One important application of biomarkers is their use to ensure that exposure limits are or have not been exceeded. To guarantee that the results obtained from biological monitoring may be correctly compared with those corresponding to the threshold limit values and also to results from other laboratories, the analysis must be carried out with reliable and validated analytical methods and must be accompanied by a quality assurance scheme. It is also important to ensure that the method of analysis be the same, or at least comparable, with that used in the literature supporting the guideline value. Confounding influences and interferences during the pre-analytical phase of new biomonitoring programs can be minimised by consultation with or recommendations from experienced laboratories (Schaller et al., 2002). Ideally, in OH practice only accredited laboratories should be utilized for BM activities (Aitio et al., 2007).

A description of several other analytical and sample collection methods is available in Human Biomonitoring for Environmental Chemicals (Committee on Human Biomonitoring for Environmental Toxicants, 2006) and in other reviews (Barr and Needham, 2002; Barbosa et al., 2005; Alexander et al., 2002; Cornelis and Nordberg, 2007).

2.4. Specific groups of biomarkers

A number of biomarkers deserve special attention due to their specific toxicological significance when monitoring specific functions or target tissues. Two such types of biomarkers will be mentioned here.

2.4.1. Biomarkers of genotoxicity

Biomarkers of genotoxicity are used to measure specific occupational and environmental exposures or to predict the risk of disease or to monitor the effectiveness of exposure control procedures in subjects exposed to genotoxic chemicals. A number of these biomarkers, for example, have been used for monitoring exposure to polycyclic aromatic hydrocarbons (PAHs), sometimes referred to as polycyclic aromatic chemicals (PACs), in a range of exposure situations from coke ovens to bitumen handling and other environmental exposures. It must be recognized that traditional genotoxicity biomarkers are generally inadequate to ORA purposes. Micronuclei and sister chromatid exchanges measured in peripheral white blood cells are also unsatisfactory as biomarkers for PAH exposure. From the relatively limited data available, however, chromosome aberrations appear to show considerable promise as indicators of exposure to/effect from PAHs (Brandt and Watson, 2003).

2.4.2. Biomarkers from “omic” technology

’Omic’ technologies include genomics, transcriptomics (gene expression profiling), proteomics and metabolomics. These new techniques are increasingly utilized in an effort to develop novel biomarkers of exposure, susceptibility and response to chemicals. ‘Omic’ technologies, for example, have a significant potential for generating novel biomarkers to monitor exposure to a number of volatile organic compounds (VOCs). Microarrays, for instance, have been applied to the study of global gene expression in the peripheral blood cells of benzene-exposed workers (Smith et al., 2005) and CYP2E1 mRNA expression has been studied in peripheral lymphocytes as a potential biomarker of effect in toluene exposed workers (Mendoza-Cantù et al., 2006). The construction and development of reliable databases that integrate information from genomic and proteomic research programmes should offer a promising future for the application of these technologies to the prediction of risks and the prevention of diseases related to chemical exposures (Watson and Mutti, 2004).

2.5. Limitations of BM studies

BM, besides its many advantages, has also some important limitations. One of them is that one cannot tell from the BM data what source the exposure originated from, e.g. whether the exposure was generated by occupational or non-occupational sources. In order to keep track of what source he/she is investigating the researcher can use questionnaires to get individual information, collect pre-exposure samples to establish baseline or background levels and/or involve 'non-exposed' controls. For occupational hygiene purposes EM is more useful than BM to detect the hazardous substances in workplaces where you can apply preventive strategies to decrease (external) exposure. In that way EM strategies can more easily lead to preventive occupational hygiene measures than BM protocols.

Biomarkers may not be sufficiently specific for assessing exposure to a particular chemical (e.g. hippuric acid is not very useful as a urinary biomarker of toluene exposure due to the high background values from diet usually found in workers). If it may not be easy to relate some exposure biomarkers to external exposure levels, it may be even more difficult to establish a relationship between exposure biomarkers and a biologic endpoint such as an adverse response or effect. Biomonitoring strategies are not useful at all if the toxic effects are local and/or acute, such as in the case of irritating agents.

The technology/methodology employed in BM research can be sophisticated. Physiologically based pharmacokinetic models (PBPK) models may illuminate subtle or unexpected results in biological monitoring and allow refinements in application and interpretation of human data. Studies on human subjects, for example, in exposure chambers, provide a wealth of pertinent information but they are subject, of course, to ethical and legal limitations. The collaboration with other disciplines has been extremely fruitful in developing early effect biomarkers (e.g. nephrotoxicity, neurotoxicity, etc.) or biomarkers that may be associated more closely with the development of pathology (e.g. neurobehavioural, reproductive, etc.). In routine use, however, there is a need for standardized, robust methodologies for comparison of test methods between different laboratories. Uniform protocols for establishing detection limits are necessary. Standardized reporting procedures and measurement units as well as a much expanded database on "normal" or reference values are all important. Availability of biological reference materials, the benchmarks of accuracy, is needed. In summary, important infrastructure that is already available in other areas of routine testing is sometimes needed for a more efficient and effective BM in OH (Draper, 2001).

3. Interpretation and management of BM data

Data interpretation and use is one of the most delicate phases of a BM program. As for any other biological or clinical test, a correct interpretation of individual or collective biomarker data requires a comparison of the results with appropriate reference values obtained in non-exposed but otherwise comparable subjects.

A multiple biomarker approach is sometimes required to integrate metabolism, temporal response and exposure–response kinetics, biological relevance, and positive predictive value. These data reinforce the notion that, for example, biomarkers relaying on carcinogen measurement are useful to monitor exposure, but a complementary approach involving effect and, when available, susceptibility biomarkers is necessary to attempt some risk assessment (Talaska et al., 2002).

3.1. Variability

Variability is an intrinsic feature of both biological and exposure measurements. Several biological and sampling/analytical sources of variability may influence biomarker levels and, therefore, taking variability factors into consideration will make the interpretation of BM data easier. In fact, it is important, when interpreting new BM data, not to "remove" biological variance but rather to uncover and explain it. In other words variability in BM may become a resource more than a limitation (Manini et al., 2007). Many of the variables that might affect BM results are actually helpful to achieve a better indication of systemic exposure. Work/breathing rates, rates of absorption (air and dermal), personal behaviour and use of protective equipment all contribute to disrupt expected correlations between BM and environmental monitoring but BM, not EM, actually reflects systemic exposure. The evaluation of the intra-individual and inter-individual sources of variation in biological measures of exposure collected on workers employed at the same plant may be essential to correctly interpret BM data. Variation among workers and variation from day to day within workers should be assessed, particularly when biological indices of exposure are used to evaluate exposure. If autocorrelation of data is undetected or ignored in the statistical analyses, the estimate of the variance components of the data will be invariably biased, thus reducing the reliability of the entire occupational risk assessment process (Symanski and Greeson, 2002).

3.2. Validity, predictive value and analytical methodology

It is becoming increasingly clear that, in order to ensure a rational occupational risk assessment, it is important to use validated biomarkers. This means that before biomarkers can be routinely used for the workers' protection they must be tested in suitable studies. It must be demonstrated that a biomarker of exposure indicates the actual exposure, a biomarker of effect truly predicts the actual risk of disease and a biomarker of susceptibility reliably suggests a modification of the risk. It must be stressed that exposure or effect biomarkers are really useful risk assessment tools when the metabolic fate of the compound (toxicokinetics) or the mechanism of a resultant disease (toxicodynamics) are completely understood. They may contribute to confusion if it is not possible to distinguish between markers of exposure, markers of disease and markers of susceptibility (Brown and Burkert, 2002; Aitio et al., 2007).

When assessing the level of exposure to a chemical, there is no simple answer to the question whether one should measure the chemical or a metabolite. Measuring the actual chemical, when applicable, may be preferable to measuring a metabolite, in order to prevent possible bias, when the same metabolites are produced by other chemicals. On the contrary, measuring the metabolite(s) may be preferable when the parent chemical is unstable or volatile, or in order to have information on its activation or detoxication metabolism or when biological limit values are to be set, particularly if the metabolite is directly involved in the mechanism of toxicity (e.g. 2,5-hexanedione in urine of subjects exposed to n-hexane). The predictive value of a biomarker may also vary with time. The stability of the sample may indeed change with the time past from sampling, unstable biomarkers being less predictive and therefore less useful than stable ones.

The predictive value of an effect biomarker is the extent to which that particular biomarker is capable of correctly separating subjects with a likelihood of impairment or disease from those without it. Validity depends on both sensitivity, i.e. the ability of the test to detect truly positive subjects as positive, and specificity, i.e. the ability to detect truly negative subjects as negative. Practically speaking it answers the following question: "How likely a person with a positive test is to have or develop a disease (sensitivity) and how likely a person with a negative test is not to have or develop the disease (specificity)?"

The predictive value mostly depends on the prevalence of the disease, on the type of chemical being measured and on the quality

of the method. With low prevalence impairments/diseases, which are in general more difficult to predict, sensitivity is more important than specificity. Whereas with high prevalence diseases, specificity is more important. Generally speaking, with prevalences in the reference population below 5% the negative predictive value of any biomarkers is high, whereas the positive predictive value is poor. The opposite occurs when the prevalence is high. Only highly specific and validated biomarkers should be used when decisions have to be made on the worker's job fitness or his/her removal from work or other important personal risk management issues, in order to avoid misjudgment, particularly with low prevalence diseases.

The extent of error in this case would be inversely related to the prevalence of disease and to the sensitivity and specificity of the test. For example, let us consider two different situations in which a test is used for assessing the workers' job fitness. Assuming that the disease has a high prevalence of 50% and the test has a low sensitivity of 80% (i.e. 20% false negative individuals) and a high specificity of 98% (i.e. 2% false positive individuals), then, out of 100 workers (50 healthy and 50 ill) undertaking the test, 41 will turn up as positive, including 40 true and 1 false positive, whereas 59 other subjects will turn up as negative, including 49 true and 10 false negative. As a result, the test would lead to the exclusion of the 40 true positive and 1 false negative workers (whereas 10 false negative workers, undetected, will continue to be exposed), with an overall, acceptable cost:benefit ratio of 1:40. Let us assume now the disease has a low prevalence of 1% and the test has a sensitivity of 99% and a specificity of 80%. Out of 100 workers examined, 21 will turn up as positive, including one only true and 20 false positive. In this case the test, if used, would lead to the exclusion of the only true positive but also of as many as 20 healthy subjects, with a cost:benefit equal to as low as 20:1. This test would clearly be ethically not justified.

3.2.1. Analytical quality

One of the most critical elements in developing a valid biomarker is the quality of the method used. This depends on a number of factors. Besides sensitivity and specificity (also called selectivity), one has to consider the following:

- the limit/range of detection, i.e. the lowest and the highest level or concentration of the chemical correctly measured by the method;
- the accuracy, i.e. the ratio of the measured value to the true value, the difference indicating the error of the measurement. Accuracy depends on the chemical to be measured, the method and the instrumentation used, and the operator;
- the precision, i.e. the level of agreement between two or more consecutive measurements of identical samples by the same methods, conditions, operator, etc.; it depends on quantitative and qualitative variability in reagents, analytical procedures (extraction, filtration, concentration, etc.) and other methodological steps;
- the reproducibility, i.e. the agreement between measurements of identical samples by the same method but under new conditions, instrumentation, operators, etc.; it depends on the overall analytical process;
- the recovery, i.e. the percent of a chemical being recovered throughout the analytical process.

Other important factors affecting the analytical quality to be considered are the time and cost of the analysis, the complexity of the methodology, the cost and reliability of the instrumentation, not to forget the experience and motivation of the operator.

The validation of a biomarker to ensure the quality of the data and permit a correct use of the results is best achieved through quality assurance (QA) programs. These involve the specific procedures, established within a quality management system, to ensure, with a satisfactory level of confidence, that a product or a test satisfies a given level of quality. QA includes the procedures for the choice of the biomarker to be used in different context, the criteria and methods for sampling and storage, the validation of the analytical methods, and also the correct documentation, interpretation and use of the results. Specific internal quality control (IQC) or external quality assurance (EQA) schemes are important additional QA tools to ensure and maintain the quality of the biomarker measurement. Although significant efforts have been made in recent years to improve the scientific quality of studies on biomarkers in occupational health, a greater attention should still be paid by those involved in BM research and practice to include QA in their studies.

3.3. Use of BM data

The practical applications of biomonitoring data to occupational exposure assessment (OEA) and ORA are almost infinite. Biological monitoring can help to assess exposure to specific chemicals, to characterize exposure pathways or to suggest potential risk factors in agricultural communities (Fenske, 2005). Biological monitoring may provide a useful tool to estimate the genetic risk deriving from an integrated exposure to a complex mixture of genotoxic chemicals. It does not provide, however, sufficient evidence for a causal relationship. Although, for instance, a positive association between occupational exposure to complex pesticide mixtures and the presence of chromosomal aberrations (CA), sister-chromatid exchanges (SCE) or micronuclei (MN) has been detected in several studies (a number of these failed to detect cytogenetic damage), the data do not allow any conclusion to be drawn on the actual genotoxic effect of exposure to the mixtures. Data available at present on the effect of genetic polymorphism on susceptibility to pesticides does not allow any conclusion either (Bolognesi, 2003). Biological monitoring, among other interventions, proved however to be effective in contributing to reduce pesticide exposure in workers (Keifer, 2000).

A number of reviews are available which report the studies on specific types of biomarkers and their role in ORA. Among them are those on male reproductive health (Ong et al., 2002), styrene genotoxicity (Vodicka et al., 2002), Comet assay in lymphocytes (Faust et al., 2004) or specific aspects of biomonitoring, such as exposure to chemical mixtures (Viau, 2002).

A few attempts have been made to establish general principles of biological monitoring of exposure to chemical mixtures. Biological Exposure Indices (BEI®) or Biological Tolerance Values (BAT) are established from the correlation between the bioindicator concentration in a given biological medium and the airborne concentration of the parent compound. When this relationship is derived from exposure to a pure chemical (as it is usually the case), the biomarker level obtained might not be suitable, in principle, to protect the workers from exposure to a chemical mixture that includes this particular chemical (Viau, 2002). In presence of significant levels of other, similar chemicals the concentration of the biomarker in the biological medium, whether blood or urine, may be substantially different than in their absence. Competition in protein binding, biotransformation or excretion, enzyme induction or inhibition and other interactions would probably modulate biomarker's level quantitatively, although the potential interferences/interactions do not usually modify the overall biomarker pattern qualitatively and, therefore, should not prevent the use of BM for ORA.

3.4. Biomonitoring and limit values

The fundamental role of biomonitoring is to assess systemic uptake or exposure and link these data to biological effects. These three elements represent the fundamental tools for a rational ORA: environmental monitoring, biomonitoring and health surveillance. Fig. 2 reports the existing relationship between these three ele-

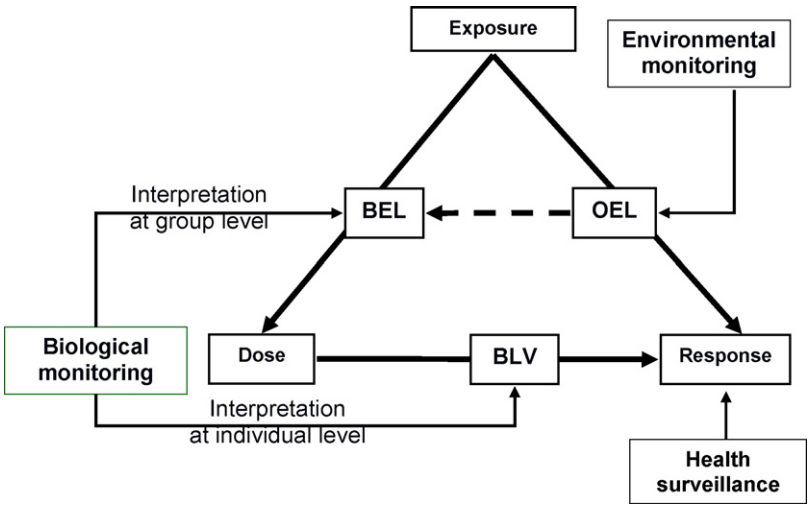

Fig. 2. Link between external exposure, internal dose and biological response and their relationship with different types of occupational limit values (BEL, OEL, BLV). Modified from the "Guidelines on Biological Monitoring" of the Italian Society of Occupational Medicine and Industrial Hygiene (Mutti et al., 2006).

ments (EM, BM and HS, respectively) and also the different types of limit or guideline values currently adopted for occupational hygiene purposes (OEL, BEL and BLV). The arrows in the scheme represent the logic direction from exposure to effect, indicating that if a limit is exceeded a possible effect may occur. Although the use of one or the other of these three types of limit values for preventive purposes is substantially similar in terms of risk management, their toxicological significance in terms of risk assessment is, or should be, quite different, at least from a theoretical point of view.

An occupational exposure limit (OEL), such as a TLV®, represents, according to the best evidence currently available, the limit concentration of a chemical not to be exceeded in the workplace in order to avoid any significant adverse effect in (the vast majority of) the exposed workers. In Fig. 2, a biological exposure limit (BEL), such as a BEI®, represents the biomarker level corresponding to, or calculated from the OEL, such as a TLV®. Therefore, although it might not be always directly associated with an adverse effect, or rather with a lack of an adverse effect, a BEL (BEI®) is used as a surrogate measure for the corresponding OEL (TLV®) and, as such, it should not be exceeded. Interpretation, however, of individual data vs. a BEI® is not always appropriate, for the very reason that a BEI® constitutively has a group nature not an individual one. In other words the observation of a biomarker level in a worker below the BEI® does not necessarily mean that the worker's exposure is by all means below the TLV® and vice versa. So, comparison of BM results with a BEI® is more reliably used for group than individual risk assessment.

A biological limit value (BLV) on the other hand represents the level of a biomarker of exposure or effect which has been directly associated with (the lack of) a biological effect or disease and, therefore, it should be considered as a somehow stronger barrier or deterrent. So, an interpretation of both group and individual data vs. a BLV is usually more appropriate than vs. a BEL. In general, for a given exposure biomarker, the dose–response relationship should be known if individual data are to be interpreted as an indication of the individual probability of an adverse effect.

A practical use of BM is to help establishing health-based occupational limits or other types of exposure limits. For example, the European Commission Directive 95/320/EC of 12 July 1995 has given the tasks to a Scientific Committee for Occupational Exposure Limits (SCOEL) to propose, based on scientific data and where appropriate, occupational limit values which may include the 8-h time-weighted average (TWA), the short-term excursion limits (STEL) and the biological limit values (BLVs). Recently, progress has been made with respect to formulation of a proper strategy related to the use of health-based BLVs (Bolt and Thier, 2006).

Various countries have published their own lists of BELs limits or BLVs. Among these, the biological tolerance values (BATs) established by the German Research Foundation and the Biological Exposure Indices (BEI®) developed by the American Conference of Governmental Industrial Hygienists represent two extensive, widely known lists of occupational exposure guidelines for use in biological monitoring. The European Union, the UK, Japan and other countries have developed their own lists of guidance values. Although there is a substantial agreement among these organizations on most basic points, there are several important differences in the approaches taken in setting the guideline values. Analysis of these distinctions may focus attention on the current issues impeding international agreement over occupational exposure guidelines. Among these issues are (1) the specification of the biological monitoring guidelines as ceiling or average values; (2) whether carcinogenic substances should be treated differently from agents with other toxic outcomes; (3) the method of accounting for variability among individual workers; and (4) the extent to which these guidelines should be extended to include specific biomarkers such as genetic markers, indicators of susceptibility, or indicators of early biological response (Morgan and Schaller, 1999).

4. Ethical considerations

Biomonitoring is one of the best, and probably the most rapidly growing tool available today for the prevention of health effects resulting from occupational exposure to chemicals. There is, therefore, a growing attention towards both the scientific and the ethical issues of biomonitoring. So the ethical and social implications of BM for epidemiologists and practitioners to consider, including issues involving individual risk estimation, the communication of epidemiologic results, and the translation of epidemiologic data into clinical or occupational health practice, have gained increasing interest by occupational physicians and within the occupational health community by and large (Schulte, 2004).

The perception of the advantages and also the limitations of this potent preventive approach may be very different among occupational health professionals, but more importantly, between health professionals and those they are seeking to protect, i.e. the workers themselves (Viau, 2005). At a time, as it is today, when the labor market is evolving towards less stable forms of work, rigid schemes attempting to adapt the workers to the workplace, referred

to as the "standardization approach" may lead to an unequal occupational health policy: the better off being more protected, the more exposed being less protected and the more susceptible being excluded. All this should be considered when planning, performing and managing the results of a BM program. In all cases, only scientifically and ethically validated biomonitoring tools should be used as part of routine medical surveillance programs (Van Damme and Casteleyn, 2003).

According to the ICOH International Code of Ethics, biomarkers must be chosen for their validity and relevance for protection of the health of the worker concerned, with due regard to their sensitivity, their specificity and their predictive value and should not be used as screening tests or for insurance purposes. Preference must be given, when possible, to non-invasive methods. When invasive tests or tests which involve a risk to the health of the worker are advisable, a risk-benefit analysis for the worker(s) concerned must be done first. In this case biomonitoring is subject to the worker's informed consent and in any case must be performed according to the highest professional standard.

Some of the most relevant ethical issues faced by those involved in BM, particularly for research purposes, are the following:

- *Planning of the study*: The first ethical requirement of any BM program is a sound scientific approach and methodology. This involves the selection of the study population, including inclusion and exclusion criteria, the validity of the biomarkers to be used, the impact of the program on individual and group risk assessment, and also a careful risk-benefit and cost-benefit analysis. It should be also made clear whether the proposed program (a) consists of a routine BM program for OEA or ORA or (b) involves epidemiological research using biomonitoring or, finally, (c) it is purely or primarily a research study on new biomarkers. The ethical approach should be different in these three cases: i.e. unnecessary, recommended and compulsory, respectively. In general, the more uncertain the toxicological significance/validity of the biomarkers to be used is, the more rigorous the ethical assessment and provisions should be. Likewise, the closer the biomarker is to the target organ and the more predictive (specific and sensitive) is the test of an adverse effect or disease, the higher will be the benefit to the worker(s) from the BM study. A detailed assessment and formal approval of the biomonitoring program by a competent ethical committee is usually required for epidemiological studies and, even more so, for human research activities involving biomarkers. For individual risk assessment or routine clinical use, however, only invasive and/or new procedures require a thorough ethical assessment.
- *Informed consent*: For research purposes or for invasive or not yet validated biomarkers, once the program has been approved as to its scientific and ethical content, but before it is actually performed, a written individual consent must be obtained, when appropriate, from each worker. This should contain adequate and clear information on the risks and also the benefits to the worker from the testing. The information should cover the meaning of the test, the sampling procedure, the interpretation of the possible results including their comparison with appropriate reference values, the forseen actions in terms of risk management, the right of the worker to withdraw at any time and the possible consequences of this in terms of job fitness. The informed consent form should be filled in by a trained OHP, preferably the occupational physician, dated and signed by both the worker and the OHP. The informed consent is not usually required for routine BM procedures with non-invasive, validated biomarkers. The ethical acceptability of monetary or other type of compensation given as an incentive to participate is, to say the least, controversial and even forbidden in some countries. In any case compensation should always be limited to incentivating tests without any significant health risk.
- *Confidentiality*: The confidentiality issue in BM should not be handled differently, in principle, that in any other OH activity. Results on biomarker testing should only be given to the worker and/or his practitioner and, when appropriate, to the competent sanitary, social security or legal authority as part of established occupational disease management programs. Individual biomonitoring data should be kept and treated as part of the worker's medical records and their use or diffusion should only be with the explicit consent of the subject involved. In research studies involving biomarkers, uncertainty in the interpretation of the biomarkers result (such as in the case of genetic, susceptibility or other sensitive tests) should not be a reason for preventing dissemination of the information to the worker involved. On the contrary, information on the difficulties and limitations in interpreting such tests correctly should be considered as an ethical priority. To the employer and the workers' representatives, only collective information should be released.
- *Communication*: The content and the form of communicating the results to the workers, individually and collectively, should be decided and agreed upon early when planning the study. The level/form of communication will be different according to the type and protocol of the study. In straightforward routine biomonitoring programs, communication of individual results (including their interpretation) to each worker and of collective results/interpretation to the employer and to the workers' representatives would be sufficient in most cases. Attention should be paid to ensure confidentiality and not to release BM data which might directly affect the worker(s) involved, his/her health or life insurance, or other sensitive personal issues. The level of communication (in terms of details, timing, interpretation, etc.) in long-term epidemiological or human research studies will depend on the type of sample, its storage, the type and duration of the study, the expected results and their possible impact on the working population and the general community. The collective results of BM studies should also be communicated and explained to the workers involved, preferably collectively. Information on risk perception by the workers, individually and collectively, should also be collected and considered but it should not be a reason to prevent but rather to improve communication.
- *Susceptibility*: A special attention should be given to the ethical aspects related with the use of susceptibility biomarkers. The critical issue here deriving from the use of susceptibility biomarkers is a correct balance between the benefit to the worker in terms of preventive action and the cost in terms of his/her possible removal from the job. In principle, BM should not result in discrimination or reduction of job opportunities for the workers involved. Another aspect of growing concern deriving from the application of new molecular biology techniques to human biomonitoring is the interpretation and use of genetic markers. Assessing the predictive value of the test correctly (including prevalence of the disease, sensitivity and specificity of the test) becomes even more important for susceptibility biomarkers than for other types of biomarkers, in order to avoid the erroneous exclusion of healthy or non-susceptible subjects (false positive individuals) or inclusion of ill or truly susceptible subjects (false negative individuals).

Ethical considerations should always be borne in mind before biomonitoring programs are to be planned and implemented, particularly when new or partially validated biomarkers are involved. Since the primary purpose of BM is the protection of the worker's health, it must be avoided that BM data, whether from exposure or effect or susceptibility biomarkers, could result in an adverse impact on the worker's status of employment and/or quality of life.

Conflict of interest

Authors have no conflict of interest.

Acknowledgements

The authors acknowledge the members of the biological monitoring scientific community which provided their comments orally and in writing. We are particularly grateful to John Cocker, Claudio Colosio, Larry Lowry, Antonio Mutti, and Monica Nordberg for their useful criticism and suggestions to the manuscript, and to Sheng Wang for providing a stimulating and enjoyable environment for discussion of the draft document during ISBM-2007 in Beijing, and to Aurélie Berthet and Caroline Couture for literature search.

References

ACGIH, 2005. Documentation of the threshold limit values and biological exposure indices. ACGIH, Cincinnati.
Aitio, A., Bernard, A., Fowler, B.A., Nordberg, G.F., 2007. Biological monitoring and biomarkers. In: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.), Handbook on the Toxicology of Metals, 3rd edition. Elsevier, pp. 65–78.
Albertini, R.J., 2001. Validated biomarker responses influence medical surveillance of individuals exposed to genotoxic agents. Radiat. Prot. Dosimetry 97, 47–54.
Alexander, J., Reistad, R., Hegstad, S., Frandsen, H., Ingebrigtsen, K., Paulsen, J.E., Becher, G., 2002. Biomarkers of exposure to heterocyclic amines: approaches to improve the exposure assessment. Food Chem. Toxicol. 40, 1131–1137.
Barbosa Jr., F., Tanus-Santos, J.E., Gerlach, R.F., Parsons, P.J., 2005. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ. Health Perspect. 113, 1669–1674.
Barr, D.B., Needham, L.L., 2002. Analytical methods for biological monitoring of exposure to pesticides: a review. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 778, 5–29.
Barr, D.B., Thomas, K., Curwin, B., Landsittel, D., Raymer, J., Lu, C., Donnelly, K.C., Acquavella, J., 2006. Biomonitoring of exposure in farmworker studies. Environ. Health Perspect. 114, 936–942.
Bolognesi, C., 2003. Genotoxicity of pesticides: a review of human biomonitoring studies. Mutat. Res. 543, 251–272.
Bolt, H.M., Roos, P.H., Thier, R., 2003. The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. Int. Arch. Occup. Environ. Health 76, 174–185.
Bolt, H.M., Thier, R., 2006. Biological monitoring and biological limit values (BLV): the strategy of the European Union. Toxicol. Lett. 162, 119–124.
Bonassi, S., Neri, M., Puntoni, R., 2001. Validation of biomarkers as early predictors of disease. Mutat. Res. 480–481, 349–358.
Boogaard, P.J., 2002. Use of haemoglobin adducts in exposure monitoring and risk assessment. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 778, 309–322.
Boumba, V.A., Ziavrou, K.S., Vougiouklakis, T., 2006. Hair as a biological indicator of drug use, drug abuse or chronic exposure to environmental toxicants. Int. J. Toxicol. 25, 143–163.
Brandt, H.C., Watson, W.P., 2003. Monitoring human occupational and environmental exposures to polycyclic aromatic compounds. Ann. Occup. Hyg. 47, 349–378.
Brown, W.E., Burkert, A.L., 2002. Biomarkers of toluene diisocyanate exposure. Appl. Occup. Environ. Hyg. 17, 840–845.
Cherrie, J.W., Semple, S., Christopher, Y., Saleem, A., Hughson, G.W., Philips, A., 2006. How important is inadvertent ingestion of hazardous substances at work? Ann. Occup. Hyg. 50, 693–704.
Clarkson, T.W., Friberg, L., Nordberg, G.F., Sager, P.R. (Eds.), 1988. Biological Monitoring of Toxic Metals. Plenum Publishing Co., New York.
Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, 2005. List of MAK and BAT Values 2005. Wiley VCH, Weinheim.
Committee on Human Biomonitoring for Environmental Toxicants, N.R.C., 2006. Human Biomonitoring for Environmental Chemicals. The National Academies Press.
Cornelis, R., Nordberg, M (2007) General chemistry of metals sampling, analytical methods and methodology including speciation analysis. In: Handbook on the Toxicology of Metals, 3rd edition (Eds.), Nordberg, G.F., Fowler, B.A., Nordberg, M, Friberg, L.T., Elsevier, pp. 11–38.
Draper, W.M., 2001. Biological monitoring: exquisite research probes, risk assessment, and routine exposure measurement. Anal. Chem. 73, 2745–2760.
Faust, F., Kassie, F., Knasmuller, S., Kevekordes, S., Mersch-Sundermann, V., 2004. Use of primary blood cells for the assessment of exposure to occupational genotoxicants in human biomonitoring studies. Toxicology 198, 341–350.
Fenske, R.A., 2005. State-of-the-art measurement of agricultural pesticide exposures. Scand. J. Work. Environ. Health 31 (Suppl. 1), 67–73 (discussion 63-5).
Gil, F., Pla, A., 2001. Biomarkers as biological indicators of xenobiotic exposure. J. Appl. Toxicol. 21, 245–255.
Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62, 806–818.
Henderson, R.F., Bechtold, W.E., Bond, J.A., Sun, J.D., 1989. The use of biological markers in toxicology. Crit. Rev. Toxicol. 20, 65–82.
Holland, N.T., Pfleger, L., Berger, E., Ho, A., Bastaki, M., 2005. Molecular epidemiology biomarkers—sample collection and processing considerations. Toxicol. Appl. Pharmacol. 206, 261–268.
Holland, N.T., Smith, M.T., Eskenazi, B., Bastaki, M., 2003. Biological sample collection and processing for molecular epidemiological studies. Mutat. Res. 543, 217–234.
Imbriani, M., Ghittori, S., 2005. Gases and organic solvents in urine as biomarkers of occupational exposure: a review. Int. Arch. Occup. Environ. Health 78, 1–19.
IUPAC (2004) Nordberg, M., Duffus, J.H., Templeton, D.M., 2004. Glossary of Terms used in Toxicokinetics (IUPAC Recommendations 2003) Pure Appl. Chem., vol. 76, No. 5, pp. 1033–1082.
IUPAC (2006), Stephenson, G.R., Ferris, I.G., Holland, P.T., Nordberg, M., 2006. Glossary of Terms Relating to Pesticides (IUPAC Recommendations 2006) Pure Appl. Chem. vol. 78, No. 11, pp. 2075–2154.
IUPAC (2007), Duffus, J.H., Nordberg, M., Templeton, D.M., 2007. Glossary of Terms used in Toxicology, 2nd edition (IUPAC Recommendations 2007) Pure Appl. Chem., vol. 79, No. 7, pp. 1153–1341.
Jakubowski, M., Trzcinka-Ochocka, M., 2005. Biological monitoring of exposure: trends and key developments. J. Occup. Health 47, 22–48.
Keifer, M.C., 2000. Effectiveness of interventions in reducing pesticide overexposure and poisonings. Am. J. Prev. Med. 18, 80–89.
Kelada, S.N., Shelton, E., Kaufmann, R.B., Khoury, M.J., 2001. Delta-aminolevulinic acid dehydratase genotype and lead toxicity: a HuGE review. Am. J. Epidemiol. 154, 1–13.
Latini, G., 2005. Monitoring phthalate exposure in humans. Clin. Chim. Acta 361, 20–29.
Lindstrom, A.B., Pleil, J.D., 2002. A review of the USEPA's single breath canister (SBC) method for exhaled volatile organic biomarkers. Biomarkers 7, 189–208.
Lucas, D., Ferrara, R., Gonzalez, E., Bodenez, P., Albores, A., Manno, M., Berthou, F., 1999. Chlorzoxazone, a selective probe for phenotyping CYP2E1 in humans. Pharmacogenetics 9, 377–388.
Manini, P., Andreoli, R., Niessen, W., 2004. Liquid chromatography–mass spectrometry in occupational toxicology: a novel approach to the study of biotransformation of industrial chemicals. J. Chromatogr. A 1058, 21–37.
Manini, P., De Palma, G., Mutti, A., 2007. Exposure assessment at the workplace: implications of biological variability. Toxicol. Lett. 168, 210–218.
Manno, M., Cocheo, V., 1989. Carboxyhaemoglobin and fatal methylene chloride poisoning. Lancet II, 748–749.
Maroni, M., Colosio, C., Ferioli, A., Fait, A., 2000. Biological monitoring of pesticide exposure: a review. Toxicology 143, 1–123.
Mendoza-Cantù, A., Castorena-Torres, F., Bermúdez de León, M., Cisneros, B., López-Carrillo, L., Rojas-García, A.E., Aguilar-Salinas, A., Manno, M., Albores, A., 2006. Occupational toluene exposure induces cytochrome P450 2E1 mRNA expression in peripheral lymphocytes. Environ. Health Persp. 114, 494–499.
Morgan, M.S., Schaller, K.H., 1999. An analysis of criteria for biological limit values developed in Germany and in the United States. Int. Arch. Occup. Environ. Health 72, 195–204.
Mutti, A., De Palma, G., Manini, P., Baccarelli, A., Bartolucci, G.B., Carta, P., Dell'Omo, M., Foà, V., Ghittori, S., Iavicoli, S., Imbriani, M., Manno, M., Perbellini, L., Pira, E., Apostoli, P., 2006. In: SIMLII (Ed.), Guidelines for biological monitoring. Maugeri Foundation Books, Pavia, p. 22.
Needham, L.L., Patterson Jr., D.G., Barr, D.B., Grainger, J., Calafat, A.M., 2005. Uses of speciation techniques in biomonitoring for assessing human exposure to organic environmental chemicals. Anal. Bioanal. Chem. 381, 397–404.
Nordberg, G.F., Fowler, B.A., 2007. Risk assessment. In: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.), Handbook on the Toxicology of Metals, 3rd edition. Elsevier, pp. 281–301.
Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.), 2007. Handbook on the Toxicology of Metals, 3rd edition. Elsevier.
NRC (National Research Council), 1983. Risk assessment in the Federal Government: Managing the Process. The National Academies Press, Washington, DC.
NRC, 2006. Human Biomonitoring for Environmental Chemicals. Committee on Human Biomonitoring for Environmental Toxicants. The National Academies Press, Washington, DC.
Ong, C.N., Shen, H.M., Chia, S.E., 2002. Biomarkers for male reproductive health hazards: are they available? Toxicol. Lett. 134, 17–30.
Phillips, D.H., 2005. DNA adducts as markers of exposure and risk. Mutat. Res. 577, 284–292.
Ross, J.H., Driver, J.H., Lunchick, C., Wible, C., Selman, F., 2006. Pesticide exposure monitoring databases in applied risk analysis. Rev. Environ. Contam. Toxicol. 186, 107–132.
Schaller, K.H., Angerer, J., Drexler, H., 2002. Quality assurance of biological monitoring in occupational and environmental medicine. J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 778, 403–417.
Scherer, G., 2005. Biomonitoring of inhaled complex mixtures —ambient air, diesel exhaust and cigarette smoke. Exp. Toxicol. Pathol. 57 (Suppl. 1), 75–110.
Schulte, P.A., 2004. Some implications of genetic biomarkers in occupational epidemiology and practice. Scand. J. Work. Environ. Health 30, 71–79.
Semple, S., 2004. Dermal exposure to chemicals in the workplace: just how important is skin absorption? Occup. Environ. Med. 61, 376–382.
Sim, M., 2002. Case studies in the use of toxicological measures in epidemiological studies. Toxicology 181–182, 405–409.
Smith, M.T., Vermeulen, R., Li, G., Zhang, L., Lan, Q., Hubbard, A.E., Forrest, M.S., McHale, C., Zhao, X., Gunn, L., Shen, M., Rappaport, S.M., Yin, S., Chanock, S., Rothman, N., 2005. Use of 'Omic' technologies to study humans exposed to benzene. Chem. Biol. Interact. 153-154, 123–127.

Symanski, E., Greeson, N.M., 2002. Assessment of variability in biomonitoring data using a large database of biological measures of exposure. Am. Ind. Hyg. Assoc. J. 63, 390–401.
Talaska, G., Maier, A., Henn, S., Booth-Jones, A., Tsuneoka, Y., Vermeulen, R., Schumann, B.L., 2002. Carcinogen biomonitoring in human exposures and laboratory research: validation and application to human occupational exposures. Toxicol. Lett. 134, 39–49.
TGMA, 1973. Task group on metal accumulation. Environ. Physiol. Biochem. 3, 65–107.
Timchalk, C., Poet, T.S., Kousba, A.A., Campbell, J.A., Lin, Y., 2004. Noninvasive biomonitoring approaches to determine dosimetry and risk following acute chemical exposure: analysis of lead or organophosphate insecticide in saliva. J. Toxicol. Environ. Health A 67, 635–650.
Van Damme, K., Casteleyn, L., 2003. Current scientific, ethical and social issues of biomonitoring in the European Union. Toxicol. Lett. 144, 117–126.
Viau, C., 2002. Biological monitoring of exposure to mixtures. Toxicol. Lett. 134, 9–16.
Viau, C., 2005. Biomonitoring in occupational health: scientific, socio-ethical, and regulatory issues. Toxicol. Appl. Pharmacol. 207 (Suppl. 2), S347–S353.
Vodicka, P., Koskinen, M., Arand, M., Oesch, F., Hemminki, K., 2002. Spectrum of styrene-induced DNA adducts: the relationship to other biomarkers and prospects in human biomonitoring. Mutat. Res. 511, 239–254.
Watson, W.P., Mutti, A., 2004. Role of biomarkers in monitoring exposures to chemicals: present position, future prospects. Biomarkers 9, 211–242.
WHO (2001). Environmental Health Criteria 222. Biomarkers in Risk Assessment: Validity and Validation. World Health Organization, Geneva.
WHO/IPCS (2006). Environmental Health Criteria 234: Elemental speciation in Human Health Risk Assessment. World Health Organization, Geneva.
Wilson, H.K., Monster, A.C., 1999. New technologies in the use of exhaled breath analysis for biological monitoring. Occup. Environ. Med. 56, 753–757.
Zielhuis, R.L., Henderson, P.T., 1986. Definitions of monitoring activities and their relevance for the practice of occupational health. Int. Arch. Occup. Environ. Health 57, 249–257.

Maurizio Manno [a,*], Claude Viau [b], in collaboration with, John Cocker [c], Claudio Colosio [d], Larry Lowry [e], Antonio Mutti [f], Monica Nordberg [g], and Sheng Wang [h]
[a] *Department of Preventive Medical Sciences, University of Naples Federico II, Via S. Pansini, 5, 80131 Naples, Italy*
[b] *Department of Environmental and Occupational Health, University of Montreal, Canada*
[c] *Biological Monitoring Section, Health and Safety Laboratory, United Kingdom*
[d] *Department of Occupational Health, University of Milan, Italy*
[e] *Health Science Center, University of Texas, USA*
[f] *Laboratory of Industrial Toxicology, University of Parma, Italy*
[g] *Institute of Environmental Medicine, Karolinska Institutet, Sweden*
[h] *Health Science Center, University of Beijing, China*

* Corresponding author. Tel.: +39 0817463838; fax: +39 0817463837.
E-mail address: maurizio.manno@unina.it (M. Manno)

Available online 13 May 2009