Review Article **Open Access**

Carina Ladeira*, Susana Viegas

Human Biomonitoring – An overview on biomarkers and their application in Occupational and Environmental Health

DOI 10.1515/bimo-2016-0003
Received August 28, 2016; accepted: October 5, 2016

Abstract: Human biomonitoring (HBM) is a scientifically-developed approach for assessing human exposures to natural and synthetic compounds from environment, occupation, and lifestyle. It relies on the measurement of particular substances or biological breakdown products, known as metabolites, in human tissues and/or fluids, and also includes the study of their effects and the possible influence of individual susceptibility as response modulators. HBM is a growing area of knowledge used for exposure and risk assessment in environmental and occupational health, and its importance has been increasing as a result of advancements in the ability to measure greater numbers of chemicals in the human body and tissues. In order to achieve this purpose, HBM focuses on the use of biomarkers as measurable indicators of early changes in biological systems. However, because data interpretation requires caution, it is strongly recommended that the interpretation of HBM results be combined with air monitoring data or pharmacokinetic modelling in order to better understand exposure sources and the metabolism of chemicals.

Keywords: Human biomonitoring, biomarkers, genotoxicity, exposure, environment, occupational.

***Corresponding author: Carina Ladeira,** Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, 1990-096 Lisboa, Portugal, E-mail: carina.ladeira@estesl.ipl.pt
Susana Viegas, Environment and Health Research Group, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, 1990-096 Lisboa, Portugal
Carina Ladeira, Grupo de Investigação em Genética e Metabolismo, Escola Superior de Tecnologia da Saúde de Lisboa – IPL, 1990-096 Lisboa, Portugal
Carina Ladeira, Susana Viegas, Centro de Investigação e Estudos em Saúde Pública, Escola Nacional de Saúde Pública, ENSP, Universidade Nova de Lisboa, 1600-550 Lisboa, Portugal

1 Introduction

Human biomonitoring (HBM) has its roots in the analysis of biological samples, aimed at looking for markers of pharmaceutical compounds and occupational exposures, in an effort to prevent the health effects of exposure to substances [1, 2]. Biological monitoring 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 [3,4]. The major goals of many research programs on biomonitoring are to develop and validate biomarkers that reflect specific exposures, and to predict the risk of disease in individuals and in population groups [5]. The power of HBM to identify spatial and temporal trends in human exposures has successfully provided exposure and risk information, in order to inform public health decisions and/or initiate policy measures, and to focus on the protection of susceptible populations, such as children and pregnant mothers [7, 8]. Classical examples are the ban of lead from gasoline, recommendations to avoid mercury-containing amalgam teeth fillings in children, the restriction of phthalate use in plastics and many others. Additionally, biomonitoring data could be used for screening purposes and prioritization of chemicals for further research or regulation [6].

Biomonitoring has many advantages over environmental monitoring; for example, biological samples reveal integrated effects of repeated exposure. Biomonitoring data directly reflect the total body burden or biological effects resulting from all routes of exposure - inhalation, absorption through the skin, and ingestion, including hand-to-mouth transfer in children - and interindividual variability in exposure levels, metabolism and excretion rates. Such data also reflect modifying influences in physiology, bioavailability, bioaccumulation and persistency, which can magnify

the concentrations of some environmental chemicals (e.g. persistent organic pollutants, and metals such as lead and cadmium) enough to raise them above detection thresholds [1,8,9]. For chemicals that are excreted rapidly, cross-sectional biomonitoring data reflect recent exposure, while characterization of long-term exposure patterns at the individual level requires repetitive sampling [9]. For a given chemical, HBM can identify spatial and time trends, lifestyle contributing factors, and specific at-risk groups. Therefore, the advantages to the individuals that participate in HBM studies include: identification of exposure (measure of body burden of a contaminant or its metabolite), identification of environmental mutagens/carcinogens, and determination of the possible range of individual susceptibility by studying genetic polymorphisms and/or pathways related to the possible injury (or causal) agent [10].

In summary, HBM of dose and biochemical effects has tremendous utility, providing an efficient and cost effective means of measuring human exposure to chemical substances and providing unequivocal evidence that both exposure and uptake have been taken place [1,8]. It can also identify new chemical exposures, track trends and changes in exposure, establish distributions of exposure among the general population, identify vulnerable groups and populations with higher exposures, and identify environmental risks at specific contaminated sites with relatively low expenditure [8].

Moreover, biomonitoring can demonstrate the association between body burden of pollutants and/or contaminants, and respective health effects in epidemiological studies merged with health data and also to test research hypotheses. Sustained national and international surveillance programs typically use well established biomonitoring techniques (e.g. biomarkers which are known to reflect exposure to the chemical of interest, standardized sampling methods and verified analytical techniques) to collect information on population exposures to environmental hazards that are known to be significant to public health [9]. Biomonitoring, however, usually does not reveal exposure sources and routes. Therefore, environmental monitoring remains crucial for the development of targeted policy actions [9].

This paper intends to make an overview about Human Biomonitoring, focusing on the use of biomarkers as biomonitoring tools, and to describe their utility and application in occupational health.

2 Biomarkers

Biomarkers have been defined by the U.S. National Academy of Sciences Committee on Biological Markers as an alteration in cellular or biochemical components, processes, structure or functions that is measurable in a biological system or sample [11], but is not a measure of the disease, disorder or condition itself [12]. A biomarker can be any substance, structure or process that can be monitored in tissues or fluids and that predicts or influences health, or assesses the incidence or biological behavior of a disease. Ideally, biomarkers should be accessible (non-invasive), non-destructive, and easy and cheap to measure. Identification of biomarkers that are on causal pathways, have a high probability of reflecting health or the progression to clinical disease, and have the ability to account for all or most of the variation in a physiological state or the preponderance of cases of the specified clinical outcome have largely remained elusive, as one is never quite sure if they fulfill such requirements [13,14].

Biological markers can contribute to quantitative risk assessment by helping to: determine the forms of dose-time-response relationships; assess the biologically effective dose; make interspecies comparisons of effective dose, relative potency, and effects; resolve the quantitative relationships between human interindividual variability; and identify subpopulations that are at enhanced risk [13]. Nowadays, most research on biomarkers is concerned with markers which will increase our ability to identify long-term risks due to toxicant exposure, in particular the risk of developing cancer, and identify early markers of toxicity in the field of environmental or ecotoxicology. Previously, biomarkers have been used to identify biological changes due to toxic chemicals and in the assessment of environmental health as part of an integrated approach. Due to the advances of molecular epidemiology, many more biological markers predictive of long-term effects will be available in the future, allowing risk assessment judgments to be made in a more accurate way [15].

The challenge in biomarker research is to facilitate the identification of environmental and genetic factors which modulate cancer risk; this challenge must be seen in the context of the fact that most environmental carcinogens appear to be associated with relative risks which are so low that they are not easily detected by classical epidemiological methods [16]. A goal in the utilization of biomarkers must be to identify adverse effects of chemical contaminants at the lowest levels of biological

organization, so avoiding toxicological problems at higher stages [15].

Biomarkers (either parent compounds or their metabolites) present a time-variable concentration profile that is associated with temporal patterns of exposure and elimination kinetics [9]. Exceptions are made for chemicals that are persistent in environment, bioaccumulate in people and/or wildlife, and are toxic (called PBTs).

The traditional, generally accepted classification of biomarkers divides them into three main categories - biomarkers of exposure, effect, and susceptibility - depending on their toxicological significance [4,13,17]. A biomarker of exposure is defined as "an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism" [4,11]. A biomarker of effect is a measurable biochemical, structural, functional, behavioral or any other kind of alteration in an organism that, according to its magnitude, can be associated with 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 [4]. 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 [4]. A further discussion of biomarkers of exposure and of effect will be provided below. Although the different types of biomarkers are considered as separate and alternative for classification purposes, 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 [4].

With respect to prevention, the use of biomarkers to quantify interindividual variability in response to exposure has significant implications for carcinogenic risk assessment and associated regulatory actions. The assumption underlying current risk assessment models - that all humans respond homogeneously to a specific carcinogen or mixture of carcinogens - is belied by the large interindividual variation observed within human populations exposed to similar levels of diverse carcinogens.

2.1 Biomarkers of exposure

Biomarkers of exposure identify and measure chemical residues in tissue or body fluids, metabolites of xenobiotic compounds, or physiological outcomes that occur as a result of exposure [9].

The fundamental role of biomarkers of exposure in occupational health practice is to assess exposure by all routes and to complement information obtained by workplace environmental monitoring. For many reasons (such as being more informative, particularly at the individual level), biomarkers of exposure are frequently used, when available, as a better substitute for environmental monitoring [4], often indicating exposures to environmental pollutants which are important to public health [8].

Exposure biomarkers can reflect bioavailability and be influenced by numerous parameters such as route of exposure, physiological characteristics of the receptor, and chemical characteristics of the xenobiotic. Exposure biomarkers have the advantage of providing an integrated measure of chemical uptake, a consideration that is important in the case of agents that exhibit large route-dependent differences in absorption [18]. Also, it is important to take in account the kinetics of biomarkers of interest, since different matrices reflect exposure over different time periods [9]. Another valuable application of exposure biomarkers is in evaluating the potential of intervention strategies. In either case, biomarkers can be used as endpoints, permitting a proof of principle to be established in advance of long-term interventions where precancerous lesions or cancer itself might be the outcome [19].

Biomarkers of exposure can be divided into markers of internal dose and effective dose. The former gives an indication of the occurrence and extent of exposure of the organism, and thus the likely concentration of a parent compound or metabolite at the target site. The simplest indicator of internal dose is the blood concentration of a chemical agent measured following exposure. The effective dose is an indication of the true extent of exposure of what is believed to be the target molecule, structure or cell. Both markers of internal and effective dose are therefore preferable to measuring external levels of the compound in question (for example in the workplace), as they take into account biological variations in absorption, metabolism and distribution of the compound between individuals [15,17]. Other examples of applications in occupational contexts are the recent publications regarding exposure to Aflatoxin B1 in several settings [20-23]. The use of an exposure biomarker demonstrates that occupational exposure is occurring in specific settings, even when exposure to this natural toxin is recognized to occur essentially through food consumption.

2.2 Biomarkers of effect

The International Programme on Chemical Safety has defined a biomarker of effect as "a measurable biochemical, physiological, behavioral or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease". This is a very broad definition. Biomarkers of effect (also known as biomarkers of response) can be elicited as a result of interaction of the organism with a host of different environmental factors (including chemical, physical, and biological agents). This definition encompasses biomarkers of effect at the level of the whole organism, at the level of organ function, at the level of tissue and individual cells, and at the subcellular level [24].

Biomarkers of effect, which measure processes of genetic damage, are sometimes used to define exposures. Because of this, classifications may be made in more mechanistic terms, such as reversible (transient) genotoxic responses (exposure/dose) and irreversible (permanent) genotoxic responses (effect) [25]. Ideally, a biomarker of effect should reflect early reversible changes in the organism. DNA adducts are better representations of penetration of the agent to the target molecules of genotoxic concern than protein adducts; however, the fact that DNA molecules are repaired (an example of reversible genotoxic response) must be considered when using DNA adducts as *in vivo* dosimeters [25]. Effect or irreversible genotoxic endpoints require host processing of DNA lesions into informational changes in the cell (e.g. mutations) and therefore may be relatively insensitive when used as dosimeters [25].

Historically and in practical terms, these biomarkers have been used most widely and routinely. They can be grouped into different categories - markers which are the result of pathological damage could be considered separately from markers which indicate a metabolic lesion [17].

Other potential uses of biomarkers of effect include monitoring of disease progression and prognosis, and as adjuncts to other biomarkers in providing refinements in epidemiology and risk assessments. Finally, biomarkers offer the opportunity to provide scientific confirmation of proposed exposure-disease pathways *in vivo* in human populations. Biomarkers of effect may be particularly useful for demonstrating the biologic influence of preceding susceptibility factors - for instance, genetic polymorphisms of xenobiotic-metabolizing enzymes [24].

This type of biomarker indicates early biochemical or functional alterations including a wide array of biological responses, ranging from physiological adaptations 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 [4].

An important group of effect biomarkers are genotoxicity biomarkers in workers exposed to mutagens or genotoxic carcinogens, which have been developed in animals (even *in vitro)* and are now increasingly applied to occupationally exposed populations,. These tests, including chromosomal aberrations, micronuclei and the more recent comet assay, 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 are difficult to interpret correctly. However, alkaline comet assays appear to be promising in distinguishing between different mechanisms of DNA damage, such as covalent binding versus oxidative stress [4].

Some of the simplest biomarkers can be very important tools in biomonitoring, as they may indicate more subtle or complex changes taking place in response to external stressors. Chromosomal abnormalities can also be identified in peripheral lymphocytes and may act as surrogate biomarkers of changes in other tissues. Micronuclei, translocations and sister chromatid exchanges, which can be induced by a wide range of exposures reflecting cumulative response to a variety of environmental factors, are also important biomarkers in this field [15,17,27]. Indeed, there are aspects of risk assessment that are best accomplished by irreversible genotoxic endpoints [25].

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 [4].

2.2.1 Biomarkers of genotoxicity

Over the past decades, biomarker-based approaches have been applied in the assessment of exposure to genotoxic agents and increases of these biomarkers are considered early events associated with disease-related changes [28]. For surrogate biomarkers to have disease predictability,

it must be demonstrated that the measured genotoxic events really mimic disease-causing genotoxic events [25].

Biomarkers of genotoxicity are considered as biomarkers of early carcinogenic effects, and are used to measure specific occupational and environmental exposures, to predict the risk of disease, or to monitor the effectiveness of exposure control procedures to genotoxic chemicals [4,9]. Cytogenetic biomarkers are the most frequently used endpoint in human biomonitoring studies, and are used extensively to assess the impact of environmental, occupational and medical factors on genomic stability [24,29]. Lymphocytes, in particular, are used as a surrogate for the actual target tissues of genotoxic carcinogens [24,26] because of the reasons mentioned above.

Genotoxicity biomonitoring endpoints such as micronuclei, chromosomal aberrations, and 8-hydroxydeoxyguanosine (8-OHdG) and DNA repair measured by comet assay are the most commonly used biomarkers in studies evaluating environmental or occupational risks associated with exposure to potential genotoxins. A review by Knudsen and Hansen [30] on the application of biomarkers of intermediate endpoints in environmental and occupational health concluded that micronuclei in lymphocytes provided a promising approach to assess health risks, but the use of chromosomal aberrations is likely to be limited by laborious and sensitive procedure of the test and the lack of trained cytogeneticists. Nevertheless, methodologies like comet assays on peripheral blood lymphocytes, urine and tissues are increasingly being used as markers of oxidative DNA damage [29,31].

2.3 Biomarkers of susceptibility

Biomarkers of susceptibility reflect intrinsic characteristics of an organism that make it more susceptible to the adverse effects of an exposure to a specific substance [9] - namely a chemical, physical or biological agent. A biomarker of susceptibility is defined as an indicator or a measure of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance [15,24]. Thus any variation in the response of an individual to identical exposures may represent some difference in susceptibility, due either to the genetic make-up of the individual or to variables and environmental influences, such as diet or the uptake and absorption of the xenobiotics [15].

Biomarkers of susceptibility are concerned with factors in kinetics and dynamics of uptake and metabolism of exogenous chemicals. Thus the concept encompasses enzymes of activation and detoxification, repair enzymes, and changes in target molecules for toxic chemicals [24, 32].

Toxicological research in experimental animals and humans over many years has revealed that individuals can often differ markedly in their qualitative and quantitative responses to chemical exposure. Such interindividual differences can be genetically mediated, or can be the result of some environmental stressor, disease process or other epigenetic factor. While these interindividual differences can complicate safety evaluation and risk assessment activities, they can also be usefully employed as biomarkers of individual susceptibility to xenobiotics [18].

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. There are two mechanisms of susceptibility to chemical agents: 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 detoxification reactions [4].

Interindividual variation occurs as a result of different genetically-inherited backgrounds, modified by dietary and environmental exposure and revealed by genotypic and phenotypic variations. Susceptibility markers are useful because they can partially explain interindividual variation inherent in the general population, and thus provide a biological rationale for investigation of inherent vulnerability prior to exposure to environmental hazards [24].

Biomarkers of susceptibility do not represent stages along the dose-response mechanistic sequence, but instead represent conditions that alter the rate of transition between the stages or molecular events. The kinetics of transition is often governed by specific enzymes or other gene products. Consequently, the determination of relative enzyme activities or the presence or absence of other gene products are often employed as susceptibility biomarkers. Enzymes involved in xenobiotic metabolism can be particularly important in the overall mechanism of action of xenobiotics, and genetic polymorphisms in metabolic enzymatic activity are a common basis for interindividual differences in toxicity [18].

An example of xenobiotic metabolizing enzymes as susceptibility biomarkers is the cytochrome P450 enzyme system. This system is responsible for oxidative (i.e. Phase I) metabolism of a multitude of xenobiotics and endogenous molecules, primarily in the liver but also in other bioactivated organs. Another important detoxification enzyme system with significant use in susceptibility studies is glutathione-S-transferase (GST), which catalyzes the conjugation (i.e. Phase II metabolism) of cellular thiol glutathione (GSH) with oxidized xenobiotics [4,18].

In addition to enzymes involved in biotransformation, other potential susceptibility biomarkers have been explored or proposed in human and animal studies. These include DNA repair enzyme activities, nuclear and cytoplasmic receptor protein levels, oncogenes and corresponding gene products, tumor suppressor genes, and humoral and cellular immune system components.

Much expectation has been vested in the development of genetic biomarkers, but for environmental and occupational fields, the research so far has not led to any routinely usable biomarkers. Many known genetic traits, such as polymorphisms of drugs metabolism, are individually only weakly-associated with disease; many probably remain unknown, due to the requirement for environmental factors. Especially if the risk of disease is associated more with exposure than with genotype, limiting exposure is the only feasible approach to prevention and benefits all [33]. However, genetic based-methods cannot easily identify all individuals at risk in a hazardous environment, due to a lack of understanding of the interaction of compensatory genetic and cellular mechanisms and complex environmental influences [24]. It is important to note that the study of genetic variations (*e.g.* from interethnic differences) associated with hazardous exposures can predict population vulnerability.

Better knowledge of xenobiotic metabolism and the pharmacokinetics of toxin elimination will speed progress of this work [24]. Possible consequences of differential interindividual and interethnic 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 the results of epidemiological or molecular epidemiological studies [4].

The use of detailed physiologically-based pharmacokinetics (PBPK) models for interpreting biomonitoring data also allows for the modelling of different sources of interindividual variability of the Absorption, Distribution, Metabolism and Excretion (ADME) process, such as body weight, age, genetic polymorphisms in xenobiotic metabolic pathways, excretion and elimination rates and others. The previously so-called confounders or uncertainty factors can be at the light of these models be treated as analyzable variables which reflect variations in the susceptibility within a population that is exposed to environmental pollutants [9].

There is also growing interest in the use and identification of "non-invasive" biomarkers, such as ones that can be found in urine, exhaled air or saliva [17]. These allow more routine sampling in human studies and may overcome ethical issues (for example, in screening children), and also increase responsiveness and compliance rates [9]. Blood is still the most commonly used and preferred biological matrix for many chemicals in HBM studies, since it is in continuous contact with the whole organism and is in equilibrium with the organs and tissues where chemicals are deposited [9, 24, 26]. HBM studies can also include analysis of environmental pollutants in other matrices, such as hair, nails, deciduous teeth, cord blood, breast milk, amniotic fluid, placenta, meconium, semen, and sweat [9].

3 Biological monitoring in occupational context

Biological monitoring has applications in exposure assessment and in occupational health surveillance programs. The term "biological monitoring" has come into use as a natural adaptation of the term "environmental monitoring", 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 [4].

When compared with environmental monitoring, biological monitoring provides additional information which can contribute for a more accurate occupational risk assessment at the individual and/or group level [4].

According to Manno et al. [4], biological monitoring of workers has three main goals: the first is individual or collective exposure assessment; the second is health protection; and the ultimate objective is occupational health risk assessment. It consists of standardized protocols aimed at the periodic detection of early (preferably reversible) biological signs, ie biomarkers 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. Biomarkers are usually more specific and sensitive than most clinical tests, and therefore may be more effective for assessing a causal relationship

between health impairment and chemical exposure when a change is first detected in exposed workers [4,10,34].

Additionally, biomonitoring can help to fill the gaps and give important information related to baseline exposure, or provide effect information needed to evaluate future exposure or health data. For instance, biomonitoring before and after exposure can establish if exposure occurred, and if health monitoring should be conducted long-term due to possible health effects arising after a long period since exposure happened [35].

Experience in biological monitoring gained in occupational settings has often been applied to assess (the effects of) human exposure to chemicals in the general environment. The use of biological fluids/tissues to assess human exposure, effects and/or susceptibility to chemicals in the workplace, together with the underlying data (e.g. personal exposure and biological monitoring measurements), represents a critical component of the occupational risk assessment process, which is a rapidly advancing science [4]. Insofar as biomonitoring data can be related to external exposures, it can provide better understanding of the relationship between health risks to external exposures, and biomonitoring can show exposures above or below the reference dose (RfD) [6]. 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 [4]. These studies should include a combination of environmental, biomonitoring, and questionnaire/survey data to assess exposures [6]. Moreover, if a substance has a sufficiently long half-life, biomonitoring tools can be used to estimate cumulative dose after repeated exposures, and can help characterize the contribution from all the possible exposure routes, such as inhalation and dermal exposure [35]. As an example, if a worker has a considerable dermal exposure due to an accident, biomonitoring will provide detailed information regarding internal exposure [35]. These studies provide a direct link between levels measured in environmental and biological media and health outcomes, allowing for an improved estimation of risks [6].

Based on the recognition that certain diseases can be caused by exposure to environmental contaminants, the movement for the prevention of environmental disease has gained broad-based public support for decades, and the public and regulatory agencies are demanding more reliable information on health risks from environmental contaminants [36]. However, we should consider that different types of biomarkers provide different information and therefore should be used with that in mind. For instance, biomarkers of exposure allow us to know the body burden related to exposure to a specific chemical, whereas biomarkers of effect would be most useful when associated with a known health outcome [35].

Overall, biomonitoring tools provide information for several actions related to occupational health interventions. Some of those are: determining if a specific exposure has occurred and if it implies a risk to workers health; providing knowledge of exposure by all possible exposure routes; realizing if health outcomes can be expected from exposure; helping to clarify the results from clinical testing in some circumstances; recognizing the adequacy of control measures in place; and helping to demonstrate the link between an occupational exposure and a health effect. Finally, the data obtained with biomonitoring tools can support health monitoring and surveillance programs, and identify possible trends in exposure [35].

There should be a trend to increase emphasis on monitoring populations which are known to be exposed to hazardous environmental contaminants, and on providing reliable health risk evaluations [36]. This information can also be used to support regulations on environmental protection and/or define limits in occupational settings. Two issues are crucial in the application of predictive biomarkers to public health policies, despite involving environmental and/or occupational exposures [9,36]. The first is dealing with the meaning of differing levels of predictive biomarkers at an individual level. A conservative and traditional approach is to consider risk predictions valid only at a group level. This interpretation allows us to cut down the effect of inter-individual variability and reduces variability due to technical parameters [37]. On the other hand, variability is a fundamental source of information. In addition, differences among individuals should not be viewed as a nuisance, but should be seen as useful hints in hypothesis generation, and as an enhanced potential to apply preventive measures in subsets of high risk subjects. The second crucial aspect of predictive biomarkers is the issue of validation. A biomarker must be validated before it can be used for health risk assessments, especially as far as regulatory aspects are involved. Despite the fact that characterization of valid biomarkers is a leading priority in environmental research, defining validity is troublesome. Validity is a general concept that refers to a range of characteristics of the biomarker, and an impressive amount of literature has been published on the concept of biomarker validity and the various aspects of the validation process [37].

The International Labour Organization (ILO) has recommended that occupational health goals for industrial nations focus on the hazards of new technology, among which pharma and biopharma products are leaders. Their unchecked growth cannot continue without a parallel commitment to the health and safety of workers encountering these "high tech" hazards. Improving the present state of affairs therefore requires: (i) recognizing healthcare as a "high-hazard" employment sector; (ii) strengthening voluntary safety guidelines to the level of enforceable regulation; (iii) conducting "potent" inspections; (iv) treating hazardous pharmaceuticals like the chemical toxicants they are; and (v) protecting health care workers at least as well as workers in other high-hazard sectors [38].

4 Limitations and social, ethical, and legal implications

The advantages of biological monitoring are countered by some important limitations. One of them is that one cannot tell from biological monitoring 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 is investigated, 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 [4]. Some biomarkers may not be sufficiently specific for assessing exposure to a particulate chemical; for instance, hippuric acid is not very useful as a urinary biomarker of toluene exposure due to high background values from diet. Therefore, relating exposure biomarkers to external exposure levels is not an easy task, and it is even more difficult to establish a relationship between exposure biomarkers and biological endpoints such as adverse responses or effects [4].

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. Therefore, there is a growing attention towards scientific and ethical issues, and social implications that must include individual risk estimation, communication of epidemiological results, and translation of epidemiologic data into clinical or occupational health practice [4].

The use of human biological samples implies special considerations, namely information consent, confidentiality, and follow-up as stated in the Declaration of Helsinki (www.wma.net). The collection of samples and personal information about health status, used for research and/or surveillance, must be preceded by a notification of the project to the ethical committee; this includes a protocol describing, for example, the risk of the persons participating, the information (oral or written) given to persons participating and the method of obtaining informed consent [5,30] (defined as consent that is informed, freely given, explicit, specific and documented) [9].

According to the International Code of Ethics, biomarkers must be chosen based on scientific criteria, namely for their validity and their relevance for protection of the health of the worker concerned, with due regard to their sensitivity, their specificity and their predictive value. Biomarkers should not be used as screening tests or for insurance purposes [4,39].

Some of the most relevant ethical issues faced by those involved in biological monitoring, particularly for research purposes, are the following: study planning, informed consent, confidentiality, communication and susceptibility [4]. The information about exposure and susceptibility gained by biological monitoring is personal and may predict health impairments. Such information may therefore be discriminative and thus sensitive in relation to future opportunities in occupational health insurance. It is therefore of utmost importance to keep all information confidential, with precise guidelines on who is allowed to use the information [30]. 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.

Finally, it is crucial that 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 [4].

The study of susceptibility in human populations poses a number of ethical challenges. Special attention should be given to the ethical aspects related to the use of susceptibility biomarkers; in particular, the benefit to the worker in terms of preventive action and the cost in terms of their possible removal from the job. In principle, biological monitoring should not result in discrimination or reduction of job opportunities for the workers involved.

The recognition of individuals who are subjected to a potentially increased risk of cancer from exposure, particularly occupational exposure, poses the ethical dilemma common to much of the present development of biomarker applications: how to prevent susceptible individuals from being exposed to these chemicals [24].

Regarding the genetic screening of workers, many critics have noted the importance of controlling workplace exposures instead of removing susceptible workers ("hyper-susceptible") from the workplace.

Ethical considerations should always be borne in mind before biomonitoring programs are planned and implemented, particularly when new or partially validated biomarkers are involved. Since the primary purpose of biological monitoring is the protection of workers' health, situations must be avoided where the data gathered from exposure, effect or susceptibility biomarkers could result in an adverse impact on a worker's status of employment and/or quality of life [4].

In summary, the strategy for data handling, data analysis, interpretation, communication, and dissemination of the results to the workers, comparison groups, and others involved in the process are all issues that should be considered in the beginning of any biomonitoring campaign [35].

5 Conclusions

HBM is a scientifically-developed approach for assessing human exposures to natural and synthetic compounds from the environment, occupation, and lifestyle. In order to achieve this, the use of biomarkers represents an integrated method of measurement of exposure to a given agent (i.e. internal dose), resulting from complex pathways of human exposure; it also incorporates toxicokinetic information and individual characteristics such as genetically-based susceptibility [40].

HBM programs provide essential information for identifying chemicals that need to be assessed with regard to potential health risks in specific population subgroups or areas [9]. It can be an important complement to the conventional sources of information for regulatory risk assessments and for supporting public and occupational health protection policies.

HBM is the only available tool that integrates exposures from all sources and provides data for epidemiological studies of association strengths, dose response relationships, and others. However, it does not differentiate the exposure by source [40]. It is important to note that HBM alone cannot provide information on how long a chemical has been in the body. It should be used in conjunction with exposure and health assessments. Linking biomonitoring data with dose, exposure, and environmental concentrations requires refined modelling tools (e.g. PBPK models, probabilistic source-to-dose models, and interfaces between exposure and PBPK models), advanced statistical approaches, and information collection tools to improve the interpretation of linkages and to reduce uncertainties [6]. Additional data should be collected (e.g., from questionnaires, interviews, etc) to provide information about potential sources, namely from patterns of dietary habits [40], hobbies and other possible confounder factors.

The analysis of HBM data related with environmental monitoring and other data of pertinent environmental sources, such as lifestyle and diet, can reveal major sources and pathways of exposure, identify risk factors and provide support to targeted interventions [9]. The implementation of standardized approaches to surveillance is mandatory to ensure international comparability of human biomonitoring data, support for policy actions and targeted interventions by identifying populations with elevated exposure levels, enabling follow-up monitoring to evaluate intervention effectiveness [9].

In summary, it is possible to conclude that HBM is useful in occupational health intervention, since it allows us to obtain detailed information about exposure and what can be expected regarding health effects resulting from exposure. Therefore, HBM can be considered an important tool for preventing exposure and exposure outcomes.

Acknowledgements: This paper is based on part of some chapters of Ladeira's PhD thesis. The authors also acknowledge WHO website that inspired the formulation of this paper as it is.

Conflict of interest: The author declares no conflicts of interest in this paper.

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