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53. Human biological monitoring and public health

Paul T.J. Scheepers

Department for Health Evidence, Radboud university medical center, Nijmegen, The Netherlands, paul.scheepers@radboudumc.nl

Abstract

Environmental exposure to xenobiotic substances can be studied by analysis of biological media such as blood, urine, or exhaled air. Uptake from different sources and via different routes is integrated over time and can be reflected in various types of biomarkers: the parent xenobiotic substance, a metabolite or the product of covalent binding to an endogenous macromolecule such as DNA or protein. The biological samples should be collected, pretreated, stored, and analyzed in a standardized manner. For interpretation of the biomarker levels, person characteristics, exposure patterns and the substances properties need to be taken into account. Such well-informed use of biomarkers is called human biological monitoring (HBM) and can be applied for different purposes: in occupational exposure surveys, exposure studies in the general populations and unexpected exposures such as in chemical incidents. The aim of an HBM campaign should be introduced to the participants with care as some sample media require invasive collection methods. Less invasive methods such as urine and end-exhaled air should be considered if they produce equal results. For interpretation, models can be used to describe the kinetics of the biomarker of interest and estimate the target dose in one or more target tissues. For answering research questions, analysis on a group level is appropriate but the results should also be made available to individual study participants, upon request.

Keywords: bioactivation, bioavailability, biological tissues, human biological monitoring, toxicokinetics, toxicodynamics

53. 1 INTRODUCTION

This chapter describes the use of biomarkers in a public health context and also indicates that this wider scope of applications of biomarkers requires much more than just the analytical challenge of determining the concentration of the substance of interest in a body tissue.

In healthcare, biomarkers are often used for diagnostic and prognostic purposes in clinical practice. Outside the hospital biomarkers are often used to study interactions of humans with xenobiotic factors from the environment. The most important difference with clinical biomarkers is that for environmental exposure and public health purposes an association with the exterior risk factors should be verifiable. The most straightforward method to achieve this is the measurement of xenobiotic substances in human tissues. In 2009 in the Zamfara region in Nigeria, 460 children died of what was assumed to be an epidemic of malaria. Blood analysis confirmed that the neurological symptoms of surviving children could be attributed to lead intoxication from exposure to lead-containing dust released during gold mining (Bartrem et al, 2013). There are many more applications of the concept of biomarkers in the public health setting as will be discussed below.

In the context of public health applications, a single biomarker level is meaningless without additional data that can support interpretation of the biomarker level (Scheepers, 2005). Each data point should be annotated with the proper contextual information that involves the person characteristics of the study subject and information on the exposure pattern. The determination of a biomarker level including this additional arrangement is described in the concept of human biological monitoring (HBM) or biomonitoring. A definition of this term was earlier postulated (Scheepers et al, 2011): The standardized and repeated systematic collection, pretreatment, storage, and analysis of body tissues in order to assess the internal dose of a xenobiotic substance by analysis of the parent substance and/or a product of biotransformation. In other words: biomonitoring is the application of biomarkers in a well-designed campaign or program aimed at answering a research question related to the impact of xenobiotic exposures on health in the general population or any specific sub population or individual.

53.2 TERMINOLOGY AND CLASSIFICATION OF BIOMARKERS

Biomarkers can be classified in many ways. A classification earlier proposed by Zielhuis and Henderson uses three main categories: biomarkers of exposure, biomarkers of susceptibility and biomarkers of effect (Zielhuis and Henderson, 1996). Examples of each of these types of biomarkers are presented in Table 53.1. Biomarkers of exposure are providing information on the uptake and systemic availability of a xenobiotic substance by representing the level of this xenobiotic substance or a product of biotransformation in the circulation.

Biomarkers of susceptibility represent parameters that reflect a person characteristic that are inherited and may also be acquired and modify the toxicokinetics and/or toxicodynamics of a xenobiotic (see section 53.4 for more details). Such biomarkers usually reflect factors that modify toxicokinetics such as bioactivation or detoxification and that can explain why a susceptible person may respond to an exposure whereas other subjects (exposed to a similar dose) do not show a response. In this way, a high activity of a bioactivating system or a low activity of a detoxifying step in metabolism may result in a higher susceptibility of an individual within an exposed population. Biomarkers of effect are a comprehensive group of markers of biochemical activity, physiological response, or effect. Most markers of effect reflect responses of a reversible nature that do not result in lesions that can be interpreted as 'adverse.' These biomarker are not necessarily good predictors of the probability of a disease to occur. There are also early indicators of a potential adverse effect that are considered biologically significant. On a population level, such effect biomarkers may explain a higher incidence or prevalence of a disease. For cancer, some biomarkers of cytogenetic damage such as chromosome aberrations have been demonstrated to be useful predictors of cancer in population-based studies (Hagmar et al, 1994; Norppa et al, 2006). Some biomarkers are not easily placed in one of the three aforementioned categories. An example is the addition product to DNA (DNA-adduct). Qualitatively, it supports a role of a particular substance in a target tissue. A good example is the DNA-adduct of benz[a]pyrene diolepoxide that supports a role of polycyclic aromatic hydrocarbons as a risk factor for lung cancer in active smoking (Garner et al, 1988). Finding this adduct in target tissue where a significant biological event is anticipated is a much stronger indication of a possible adverse outcome than just the presence of a parent xenobiotic substance in a target tissue. It demonstrates that the substance was taken up and distributed to that particular tissue. In addition, it demonstrates that the substance was bio-activated to produce a reactive intermediate capable of forming a covalent bond to a biomolecule with a critical role in human physiology. As DNA is a critical cell

component, there are several biochemical mechanisms to prevent changes in the chemical structure of DNA, and (as relevant in this case) it also contains very effective systems to remove structure modifications that may thus have an adverse effect. Most adducts will therefore disappear within days or weeks due to enzymatic DNA-repair. The adduct level may not be a good predictor of the health outcome (tumor induction): this is much (more) dependent on how effectively the adduct can be removed by enzyme repair.

53.3 BODY TISSUES

In the field of HBM, the term body tissue as a medium of sample collection is widely interpreted to include organ tissue, blood, and urine but also exhaled air, saliva, buccal smears, hair, and nails.

53.3.1 Organ tissue

There are limited possibilities for collecting samples from body tissues. Since skin, lung, and gastrointestinal tract represent a tissue that is in direct contact with the exterior and forms a first line of defense, these organs may be relevant for studying the local bioavailability and toxification or detoxicification in target tissues. Cells in direct contact with xenobiotics can be sampled by taking biopsies from skin (Roelofzen et al, 2012) or cells may be flushed, e.g., from the lung lumen, i.e., by a bronchial alveolar lavage or sputum (Talaska et al, 1996). Such procedures are invasive and must be performed with great care because of potential risk to the study subject. A less invasive method is the collection of epithelial cells from buccal smears or exfoliated cells from urine (Talaska et al, 1993). Both procedures are less invasive and also provide epithelial tissues, which may be targets for specific chemicals.

53.3.2 Blood

Collection of venous blood provides the possibility to study the systemic availability of a xenobiotic substance following absorption from different routes of uptake (inhalation, dermal absorption, and absorption via the gastrointestinal tract).

Table 53.1 Classification of biomarkers, which covers not all but most of its applications.

Class	**What it is**	**What it describes**	**Example**	**Reference**
Biomarker of exposure	Parent substance or a product of (usually covalent chemical) interaction with an endogenous biomolecule	Related to a xenobiotic substance and reflecting a systemic internal dose in tissue relevant to the primary target organ or tissue	Blood lead	Skerfving et al, 1993
Biomarker of susceptibility	An enzyme or enzyme activity or signal in the pathway between exposure and effect	Related to a constitutional property (genotype) attenuated by acquired characteristic (phenotype) that modifies the response of the physiology to a xenobiotic exposure	Activity of isoforms of enzymes systems, such as cytochrome P-450 isoenzymes (CYP), glutathione-S-transferase (GST), acetyltransferase, and UDP-glucuronosyl-transferases	Kadlubar et al, 1992; Autrup, 2000
Biomarker of effect	Any physiological change in structure or function of bodily constituents that can be interpreted as related to or leading to a potential or proven adverse event	Related to response of the body physiology that is not necessarily adverse but contains information on a biologically significant interaction of a xenobiotic factor with critical tissues or processes	Chromosome aberration	Hagmar et al, 1994; Norppa et al, 2006

Blood by itself represents a complex biological medium due to the presence of different cell populations that may contain target biomolecules such as DNA, RNA, and proteins, and also active metabolic pathways. Once a xenobiotic substance or its product of metabolism is taken up in a cell, the kinetics of elimination may be changed (see section 53.4). Even without uptake in blood cells, the bioavailability of a xenobiotic substance may be influenced by protein binding in blood and organs and by partitioning between the blood circulation and perfused tissues. Because of the changes in bioavailability and metabolism over time, a plasma level can be an important source of information, especially if acute health effects are to be expected and treatment is considered (Chapter 54). Blood parameters reflect exposure but also bioavailability and, for xenobiotic species that require bioactivation, the cytochrome P-450 (CYP) enzyme system represents a comprehensive system of different iso-enzymes responsible for conversion of many substrates including xenobiotic substances. Many such conversions may lead to inactivation and rapid conjugation and elimination of toxic species but in some cases, such as polycyclic aromatic hydrocarbons (PAH), metabolites have a higher chemical reactivity and toxicity (Fig. 53.1).

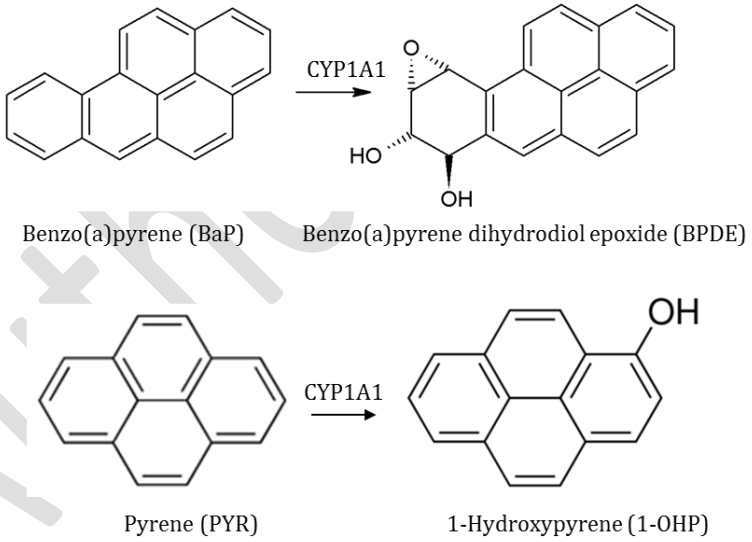

Figure 53.2 Bioactivation of two congeners of polycyclic aromatic hydrocarbons (PAH) and their respective metabolites that are often used as biomarkers of exposure to PAH.

For PAH reactive intermediates formed by CYP enzymes, adducts can be used as biomarkers of exposure. PAH epoxides represent the reactive intermediates responsible for covalent binding to nucleophilic groups in biomolecules such as DNA (Fig. 53.3).

For those substances that form highly reactive intermediates, the most useful biomarkers may be DNA-adducts from peripheral blood lymphocytes, albumin adducts in plasma, or hemoglobin adducts in erythrocytes. DNA-adducts have a limited half-life due to their repair, which normally takes a few days. For proteins, there is no known system of enzymatic repair and these adducts are therefore more persistent and depend on the life span of the native protein. Chemically stable adducts of hemoglobin (Hb-adducts) have a persistence equal to the lifespan of the erythrocyte, which is four months in humans. Analytical methods have been developed for many different biomarkers based on Hb adducts (Scheepers et al, 2009, see Table 53.2). For specific xenobiotics, blood biomarkers may be used to assess exposure and potential health risk, e.g., formation of methaemoglobin by amines and nitro-compounds, formation of carboxyhemoglobin by exposure to carbon monoxide or dichloromethane, and inhibition of acetyl cholinesterase from erythrocytes by organophosphor pesticides or carbamate pesticides and nerve agents. Porfyrine assays have been developed to assess the blood toxicity of different hazardous substances such as lead. Blood is also used to evaluate the toxicity of cytogenetic damage (Comets, micronuclei, sister chromatide exchanges, and chromosome aberrations). These cytogenetic markers demonstrate molecular lesions that reflect systemic bioavailability and bioactivation. Some of these parameters reflect early biological responses that may be indicative of a potential risk if exposure extends over long periods (years) of time. For chromosomal aberrations, an association with cancer was suggested (Hagar et al, 1994; Norppa et al, 2006).

53.3.3 Exhaled air

The lungs effectively exchange gases providing bodily tissues with oxygen and removing excess carbon dioxide. In addition to oxygen, other substances can be taken up by this route and in addition to carbon dioxide many other gases and vapors are excreted via the lungs. Due to the large surface and the short distance between the blood and air compartment in the alveoli, low-molecular substances in the gas phase equilibrate in a matter of milliseconds. The uptake and excretion are therefore ruled by the blood gas partition coefficient of any gas or liquid with a vapor pressure. The mixture of gases and vapors in the alveolar air volume will reflect the blood composition with respect to gases and volatile liquids. The relationship between alveolar air concentrations and arterial blood concentrations is known to be linear over a wide concentration range. This knowledge is used in the breath alcohol test to determine the blood percentage of drivers and can be applied to many other volatile organic

compounds in industrial and consumer products. Substances that have low vapor pressure, such as most metal ions, can be captured from exhaled breath condensate and represent suitable noninvasive biomarkers of pulmonary exposure (Félix et al, 2013).

53.3.4 Urine

Most nonvolatile water soluble inorganic and organic xenobiotic substances are readily excreted in urine. In addition, traces of non-metabolized volatile organic compounds can be determined in exchange with the gas phase of urine (Fustinoni et al, 1999). Urine is continuously formed by the kidneys and collected in the bladder. If urine samples are required, it is useful to provide detailed instructions of how and when to collect the samples. Depending on the time and duration of exposure, it may be sufficient to collect only one urine sample (referred to as spot-sample) at a well-chosen time of the day (e.g. a first urine sample after awakening). If the exposure event cannot be pinpointed in time, it may be required to collect a number of urine samples over a defined period of time following an exposure event. Information on the time of the toilet visit (and the previous toilet visit) provides information on the period of time when the biomarker was excreted in the bladder. For standardization, it may be required to define the moment of sample collection, such as in workers (pre-shift and post-shift spot samples), or to collect urine of a defined period of time, i.e. a whole day and night (24 h sample). There is quite some variability in the density of urine samples due to the intake of beverages and loss of water vapor during exercise and/or in a warm climate. The creatinine adjustment is the most common approach to correcting for this variability (Garde et al, 2004). The half-life of urinary biomarkers is usually short but may be attenuated if there are sinks of poor water soluble depots, e.g., in the lungs (Scheepers et al, 2008) or due to binding to metallothioneins (metals).

53.3.5 Saliva, hair, and nails

Saliva is sometimes used as a biological medium for environmental exposure monitoring (Nigg and Wade, 1982; Luconi et al, 2001). Hair and nails have been used to estimate exposure to metals (Kim and Kim, 2011). The content of mercury in hair was used to reconstruct an intoxication in the general population in Iran (Crump et al, 1995). Recently, also organic substances have been determined from hair (Mercadante et al, 2013).

Table 53.2 New and improved biomarkers of exposure that were presented over the past 10 years (Scheepers and Heussen, 2008; Scheepers,2011; Scheepers et al, 2013).

Chemical substance	**Biomarker**	**Biological medium**	**Method[a]**
Acrylonitrile	N-Acetyl-S-(2-cyanoethyl)cysteine (CEMA), N-acetyl-S-(1-cyano-2-hydroxyethyl)cysteine (CHEMA)	Urine	LC-MS/MS
Arsenic species	Arsenobetaine (AB), arsenite (As^{3+}), Arsenate (As^{5+}), dimethylarsinate (DMA), monomethylarsonate (MMA)	Urine	µLC-ICP-MS
Benzene and toluene	S-phenyl mercapturic acid (SPMA) S-butyl mercapturic acid (SBMA)	Urine	LC/LC-ESI-MS/MS
Benzene	S-phenylmercapturic acid (SPMA)	Urine	ELISA
	Benzene	Urine	SPME-GC-MS
Benzene, toluene and xylene-isomers	Benzene, toluene, ortho-xylene, para-xylene and meta-xylene	Exhaled air	GC-FID
Beryllium	Beryllium	Urine	Quadrupole ICP-MS

Bis(2-propylheptyl)phthalate (DPHP)	Mono-2-(propyl-6-hydroxyheptyl)phthalate (OH-MPHP), Mono-2-(propyl-6-oxoheptyl)phthalate (oxo-MPHP), mono-2-(propyl-6-carboxy-hexyl)phthalate (cx-MPHxP)	Urine	LC-MS/MS
Ethylene oxide	N-Acetyl-S-(2-hydroxyethyl) cysteine (HEMA)	Urine	LC-MS/MS
Nicotine	Cotinine (COT), trans-3'-hydroxycotinine (HCOT)	Urine	Online-SPE-UPLC-MS/MS
Decamethylcyclopenta-siloxane (D5)	Decamethylcyclopentasiloxane (D5)	Exhaled air	TD-GC-MS
1,1-difluoroethane, 1,1,1-trifluoroethane, 1,1,1,2-tetrafluoroethane, 1,1,1,3,3-pentafluoropropane	1,1-Difluoroethane, 1,1,1-trifluoroethane, 1,1,1,2-tetrafluoroethane, 1,1,1,3,3-Pentafluoropropane	Exhaled air, blood	GC-FID, ATD-GC-FID
N,N-dimethylacetamide and N,N-dimethylformamide	N-Methylacetamide and N-methylformamide	Urine	GC-MS
N,N-dimethylformamide (DMF)	3-Methyl-5-isoproylhydantoin (MIH, released from N-terminal Hb adduct), N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC)	Blood, urine	GC-MS (MIH), LC-MS/MS (AMCC)
Dithiocarbamates (DTC)	Ethylenethiourea (ETU), propylenethiourea (PTU)	Urine	UHPLC-ESI-MS/MS

2-ethoxyethanol	2-Ethoxyacetic acid (EEA)	Urine	GC-MS
N-ethyl-2-pyrrolidone (NEP)	5-Hydroxy-N-ethyl-2-pyrrolidone (5-HNEP); 2-hydroxy-N-ethylsuccinimide (2-HESI)	Urine	GC-MS
Ethylenethiourea	Ethylenethiourea	Urine	LC-ESI-MS/MS
n-Heptane	n-Heptane and its metabolites heptane-2-one, heptane-3-one, heptane-4-one, 1-heptanol, 2-heptanol, 3-heptanol and 4-heptanol	Urine	HS-SPDE and GC-MS
1,6-Hexamethylene diisocyanate	1,6-Hexamethyl diamine	Urine	GC-MS
Lead	Lead	Saliva	ICP-MS
Methamidophos	Methamidophos	Urine	LC-MS/MS
Naphthalene	Naphthyl-keratin adduct	Blood	ELISA
Octamethylcyclotetrasiloxane (D4)	Octamethylcyclotetrasiloxane (D4)	Exhaled air	TD-GC-MS
Polycyclic aromatic hydrocarbons (PAH)	1-Hydroxypyrene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 2-hydroxyfluoranthene	Urine	HPLC-Flu
Polycyclic aromatic	1, 2-hydroxynaphthalene, 2-, 9-hydroxyfluorene, 1-, 2-, 3-, 4-, and 9-	Urine	GC-MS

hydrocarbons (PAH)	hydroxyphenanthrene, 1-hydroxypyrene, 6-hydroxychrysene and 3-hydroxybenzo[a]pyrene, naphthalene, acenaphtylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[a]pyrene		
Pirimicarb	2-Methylamino-5,6-dimethyl-4-hydroxypyrimidine (MDHP)	Urine	LC-MS
(1S,6R)-(+)-3-carene	3-Caren-10-ol, 3-caren-10-carboxylic acid	Urine	GC-PCI-MS/MS
(1R,5S)-(+)-α-pinene, (1S, 5S)-(-)-α-pinene	(1R,2R,5R)-cis-verbenol, (1R,2S,5R)-trans-verbenol, (1S,5R)-(+)-myrtenol	Urine	GC-PCI-MS/MS
(R)-(+)-limonene	(1S, 5S)-trans-Carveol, (1R, 5S)-cis-carveol, (1S, 2S, 4R)-limonene-1,2-diol, perillyl alcohol , perillic acid, limonene-8,9-diol	Urine	GC-PCI-MS/MS
4,4'-methylenediphenyl diisocyanate (MDI)	5-isopropyl-3[4-(4-aminobenzyl)phenyl]hydantoin	Blood	GC-HRMS-NICI
Isocyanurate	Isotriamine	Urine	UPLC-MS/MS
Indium	Indium	Serum	ICP-MS
2- and 3-Nitrobenzanthrone	2-Aminobenzanthron-3-ylmercapturic acid (2-ABA-MA), 3-aminobenzanthron-3-ylmercapturic acid (3-ABA-MA)	Urine	LC-ESI-MS/MS

PAH	Parent polycyclic aromatic hydrocarbons	Urine	SPME-GC-MS
Phenyl urea herbicides	Parent phenyl urea herbicide, methyl urea, urea and aniline	Urine	LC-MS/MS
Styrene	S-(2-hydroxy-2-phenylethyl)cysteine adduct to globin	Blood	GC-EI-MS
Styrene	Styrene	Saliva	SHS-GC-MS
Tebuconazole	t-Butylhydroxy-tebuconazole (TEB-OH), t-butylcarboxy-tebuconazole (TEB-COOH)	Urine	LC-MS/MS
Terbuthylazine	Terbuthylazine (TBA), desethylterbutylazine (DET)	Urine, hair	LC-MS/MS
2,5-Toluylenediamine	2,5-Toluylenediamine	Urine	GC-MS
o-Tricresyl phosphate	o,o-Dicresylphosphate	Urine	GC-MS/MS
Bifenthrin/Cyhalothrin	3-(2-Chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (TFP-acid);cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propanecarboxylic acid (cis- and trans DCCA); cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DBCA); 4-chloro-isopropyl benzeneacetic acid (CPBA); 3-phenoxybenzoic acid (3-PBA); 4-fluoro-3-phenoxybenzoic acid (F-PBA); 2-methyl-3-phenylbenzoic acid (2-MPA)	Urine	GC-MS/MS

[a] ELISA = enzyme-linked immunosorbent assays; GC-NCI-MS = gas chromatography negative chemical ionisation mass spectrometry; GC-PCI-MS = gas chromatography positive chemical ionisation mass spectrometry; GC-HRMS-NICI = gas chromatography high resolution mass spectrometry negative ion chemical ionisation; ICP-MS = inductively coupled plasma mass spectrometry; SHS-GC-MS = static head space gas chromatography mass spectrometry; SPME = solid phase micro extraction; µLC = micro liquid chromatography; UPLC-MS/MS = ultra-performance liquid chromatography tandem mass spectrometry; µLC-ICP-MS = micro liquid chromatography inductively coupled plasma mass spectrometry; UHPLC-ESI-MS/MS = ultra high performance liquid chromatography electrospray ionisation tandem mass spectrometry.

53.4 TOXICOKINETICS AND TOXICODYNAMICS

A popular expression to describe the contribution of toxicokinetics is: *what does the body do to the substance* as opposed to the contribution from toxicodynamics: *what does the substance do to the body*. Toxicokinetics describe the absorption, distribution, metabolism, and excretion of xenobiotics. These processes describe whether, when, and at what concentration a xenobiotic or one or more of its metabolites may reach a target tissue where it can do harm. For uptake, three routes have to be taken into account: inhalation, ingestion, and skin absorption. Substances can be taken up by only one of these routes, two of them, or all of them, depending on the substance properties and exposure situation. Inhalation is often fast, ingestion may be fast in most cases, and skin absorption is fast for only a few substances. For distribution, the partitioning between air and blood, between blood and organs, and between blood and adipose tissue is important. The lipophilicity expressed as the partitioning between octanol and water (Log $P_{o/w}$) is a useful parameter to predict the kinetics of uptake, distribution, and excretion. For some target tissues, such as the brain and the placenta, it is useful to know whether and to what extent a xenobiotic or its metabolite can pass. If a substance can be taken up efficiently, is taken up in the blood circulation, and can reach the target organ, it is said to have a high bioavailability. Most organic substances are metabolized involving enzyme-systems that protect vital systems. Such enzyme systems can be found in all tissues but some organs such as the liver may be more important to overall metabolism because of higher concentrations or activity of enzymes or higher concentrations of substrates. For ingestion, the liver is the first organ to contribute to metabolism. If substances are taken up via inhalation or skin, the parent may reach other organs before passing through the liver or may be metabolized in lungs or dermis. There may be differences in iso-enzymes available in different organ systems and this may have consequences on the tissue dose of the parent and of one or more metabolites (Kadlubar et al, 1992).

53.4.1 Zero- and first order kinetics

For the study design of HBM campaigns, it is useful to know what kinetic pattern the biomarker of interest will follow. In this respect, the distinction between zero– and first order kinetics is the most relevant. First order kinetics applies to xenobiotics and their metabolites that are free in solution and dissolved in plasma and other body fluids. The half-life of excretion is determined by the overall effect of kinetic processes (see Fig. 53.1 for the

influence of elimination half-life on the pattern of excretion of the biomarker). In contrast, zero-order kinetics applies to xenobiotics and their products of metabolism that are formed in the cell interior and that cannot leave the containment of the cell.

This is the case for chemically stable addition products (adducts) of reactive intermediates with intracellular structures and biomolecules, such as DNA, RNA, and proteins. The half-life of these biomarkers is determined by the lifespan of the cell that contains the biomarker of interest. For white blood cells, this depends on the lifespan of the specific subpopulation of the cells. For red blood cells, this lifespan in humans is 126 days. The relative pattern of biomarker elimination over time is shown in Fig. 53.1. Such kinetic patterns can be measured and modelled. In practice, toxicokinetic models are often validated using data from analysis of body tissues and used for predictions of changes of concentrations in different body tissues over time (see 53.5.3). Kinetic information such as the pattern of elimination and the elimination half-life can already be used in the planning phase, e.g., to calculate the time available to collect a sample, regarding the anticipated type of elimination kinetics and the biological half-life $t_{½}$ of the specific biomarker. For biomarkers that follow zero kinetics (see 4.1), the time t_s available to collect a sample following an exposure event is equivalent to the estimated population-based life span of the cell that contains the biomarker. This life span is twofold the elimination half-life of the biomarker, only if the limit of quantification (*LOQ*) is much lower than the concentration of the biomarker in the available biological medium estimated at the time of exposure, C_e (Scheepers et al, 2011): $t_s \approx 2 \cdot t_{½}$ if $LOQ << C_e$

For biomarkers that follow a first order pattern of elimination (see 4.1), the time t_s to collect an air sample is equivalent to (Scheepers et al, 2011): $t_s = t_{½} \, ^2\log(C_e/LOQ)$

with $t_{½}$ as the elimination half-life of the biomarker of interest in a biological medium that is available for sample collection.

53.4.2 Modifying factors

There are some person characteristics that should be considered when interpreting biomarker levels. In this section, the influence of gender and age, physical activity, and co-exposures will be discussed as some examples of factors that can modify biomarker levels quite substantially.

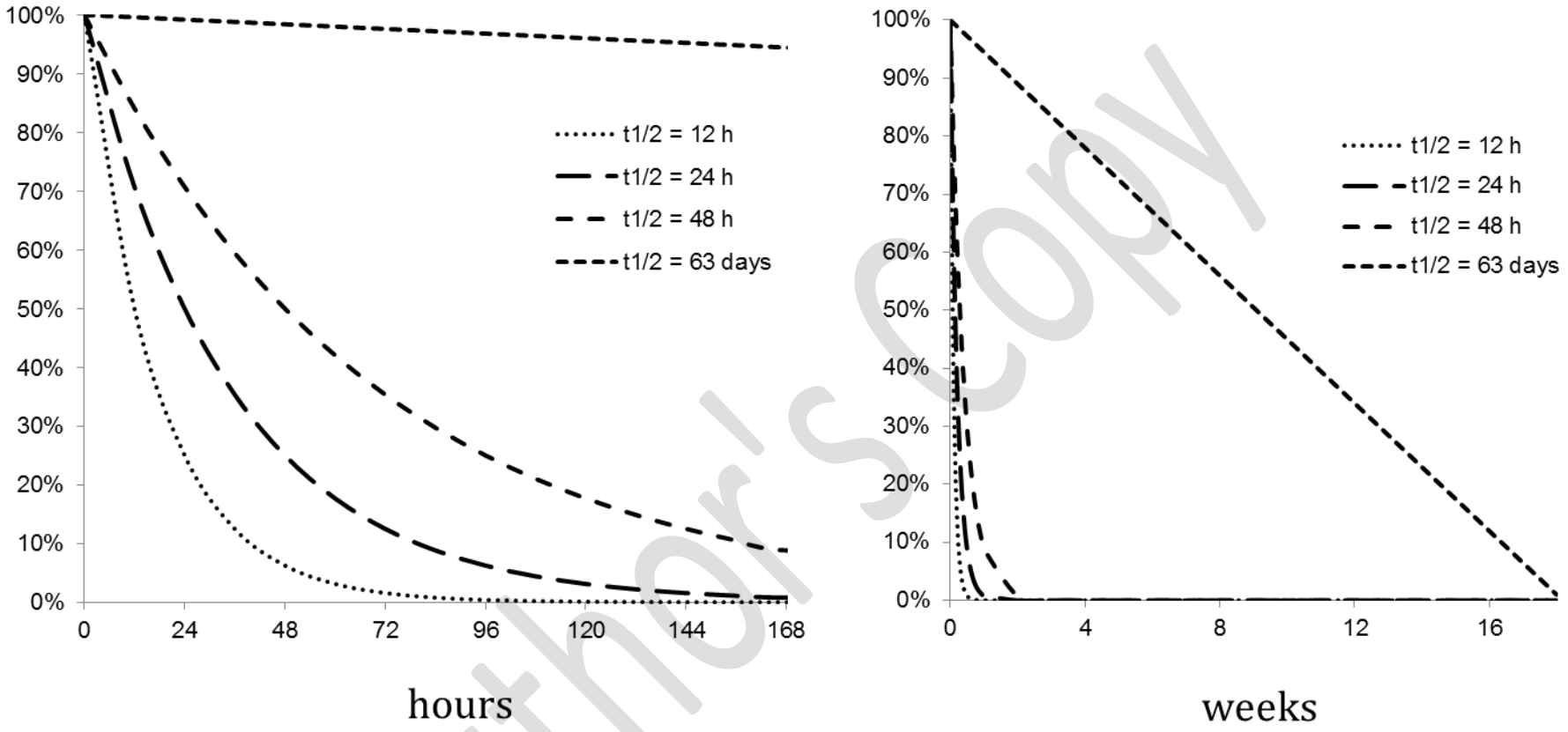

Figure 53.1 Zero and first order elimination of biomarkers with different half-lives. The same data are shown over a time course of hours (left panel) and over the time course of weeks (right panel).

53.4.3 Gender and age

Physiology in males and females is different. This relates to the distribution of adipose tissue and to differences in some hormone-directed processes. Young children are likely to have higher exposures than adults. In part, this is related to their body biometry and body physiology (body length and weight, high surface to volume ratio, somewhat higher lung ventilation frequency, etc.). Also, their behavior and activity patterns may attenuate internal exposures (e.g., personal hygiene, hand-mouth contact, sucking and licking of toys and other objects, etc.). It is obvious that it is relevant to register gender and age together with other useful person characteristics.

53.4.4 Physical activity

Physical activity has a profound influence on the ventilation frequency and tidal volume. This may result in an increased uptake rate of xenobiotics from the gas phase. Post-exposure elimination of volatile xenobiotics or their metabolites by inhalation will also be much faster at an elevated level of physical activity due to a higher ventilation rate. A higher physical activity also results in an increased cardiac output that will enhance the distribution to and perfusion of organs. An increased liver perfusion will likely result in a much faster metabolism of xenobiotics (Jonsson et al, 2001). Obviously, when studying exposures of workers it is useful to determine and register their level of physical activity during different phases (pre-exposure, exposure, and post-exposure).

53.4.5 Co-exposure

Most exposures are not to single substances. In addition there may also be life style factors such as smoking of tobacco, drinking of alcoholic beverages, or other uses of stimulating or sedating substances. A particular interesting co-exposure is the use of prescribed or non-prescribed medical drugs. All of these exposures may interact with the toxicokinetics and the toxicodynamics of the substances of interest. Competitive inhibition of enzymatic conversions by medical drugs may alter metabolism (Campbell et al, 1988). Some other co-exposures provide substrates to an enzyme system, increasing the metabolic rate for the xenobiotic of interest. When applying biomonitoring, it is useful to keep track of these co-exposures by asking the study participants some specific questions related to their lifestyle and their use of medication.

53.4.6 Modelling

To some extent it is possible to simulate the toxicokinetics and toxicdynamic processes in a model. As long as exposures are low, most processes can be described in simple toxicokinetic models (TK-models) using linear differentiation equations. If exposures are higher, biotransformation pathways may become over-saturated resulting in more complex processes that require models based on more complex nonlinear differential equations. More accuracy is obtained using models that more precisely simulate human physiology in so-called physiology-based toxicokinetic (PBTK) models. Many substance and body-specific parameters have to be inserted before biomarker levels can be calculated. Once parameterized, the course of biomarker levels over time can be estimated for specific target organs by calculation. The recent introduction of generic PBTK models simplifies the use of these models in spread sheet-based software applications (Jongeneelen et al, 2011a, 2011b). Such models could be used to derive biomarker levels to protect workers or the general public (section 53.5.2). Another interesting application is the use of such models in reverse dosimetry related to chemical incidents. During follow-up to a high exposure, biological tissues are often available for analysis. Because biomarker levels change over time, it is useful to reconstruct the exposure level at the time of the incident. This information can be used in the medical support, for risk assessment, or for scientific research purposes (see chapter 54).

53.5 INTERPRETATION AND COMMUNICATION

The concentration of a parent, metabolite, or adduct provides information on the uptake of xenobiotics, taking into account the overall contribution from different routes of exposure and from different sources of the xenobiotic substance. The level may vary over time and reflects the systemic uptake integrated over different toxicokinetic processes in time. Without additional information on the study subject's person characteristics (gender, age, biometry, physical exercise, co-exposures, etc.), and about the exposure pattern, it is hardly possible to derive useful information from a biomarker concentration concerning the exposure or potential health effects (Edelman et al, 2002).

53.5.1 Individually and in groups

Results of a biomonitoring campaign can be presented in descriptive statistics. If required, the results can be stratified according to gender, age, or anticipated exposure status. Stratification for life style factors such as smoking and drinking habits is recommended even if these co-exposures may not contain the xenobiotic of interest, as smoking and drinking of alcoholic beverages can attenuate the biomarker levels substantially by changing toxicokinetics such as enzyme activities (see 53.4.5). Use of prescribed medical drugs may also interfere with toxicokinetics or toxicodynamics of the xenobiotic and such effects should also be treated in a separate analysis if possible.

53.5.2 Values for reference

The most obvious reference level is a baseline level observed for the biomarker in the population of interest. A baseline is defined as the biomarker level observed when there is no known specific exposure other than background exposure. Such a baseline is determined by person characteristics and life style factors (VanRooij et al, 1994). Large population-based HBM programs were introduced in the US and Europe to describe the levels of biomarkers reflecting common and ubiquitous environmental contaminants (Kirman et al, 2012; Joas et al, 2012). If such values are not available for the population of interest, it is possible to determine the baseline by collection of samples before the start of a known exposure event. In populations of workers, a Monday morning sample, prior to the start of a work-shift, gives a suitable reflection of a baseline as long as the half live of elimination is sufficiently short and any traces from a previous workweek were eliminated. Biomarker levels in follow-up spot samples can be interpreted relative to this individually determined reference. For substances with fast toxicokinetics, the comparison of post-shift with pre-shift values is a suitable approach to identifying work-related factors as potential sources of uptake. For biomarkers with a much slower pattern of excretion, samples may have to be collected over several days up to a full workweek because of potential accumulation of the biomarker level over a workweek. The biomarker level in a post-shift sample on the last day of a workweek may provide a value that can be compared to a workplace standard, such as a biological limit value (BLV), for workers. BLVs are specifically established for the desired type of biological sample, as well as the time of sample collection, relative to a period of exposure. BLVs have been published in the US, UK, Finland, and Germany (see for an overview Boogaard, 2009).

Most BLVs have been derived from maximum allowable air levels determined to protect worker's health (Bevan et al, 2012). Equations describing the numerical association between levels of inhalation exposure and biomarker levels have been published by the Deutsche Forschungsgemeinschaft (DFG, 2012).

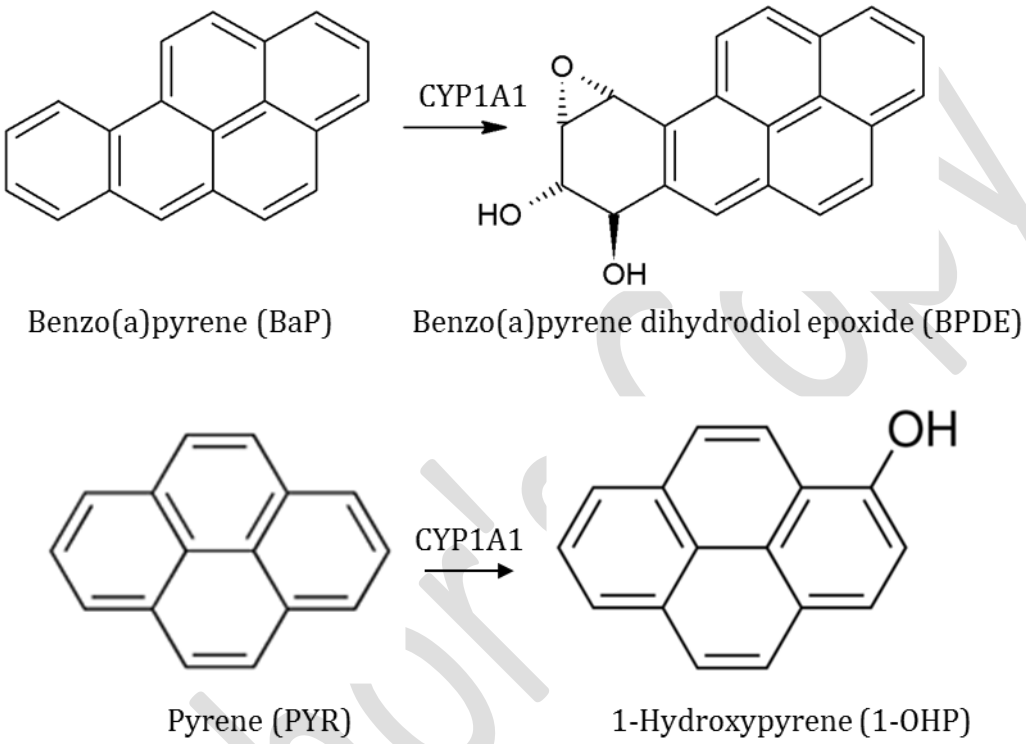

Figure 53.2 Bioactivation of two congeners of polycyclic aromatic hydrocarbons (PAH) and their respective metabolites that are often used as biomarkers of exposure to PAH.

53.5.3 Pattern over time

Following the end of exposure, biomarker levels will normally gradually decrease towards background levels that are considered to be a baseline within a population (see 4.1). If the levels remain the same or go up in the post-exposure phase this may indicate a latency in uptake into the circulation from a depot in, e.g., skin, lungs, or intestine. Since skin absorption is a slow process compared to inhalation and gastrointestinal absorption, latency in skin absorption may cause the highest plasma concentration to be reached several hours following cessation of exposure. For substances with high acute toxicity, it is recommended that admission to a healthcare facility be considered because symptoms of intoxication may occur

a considerable time after the exposure ended (see chapter 52). If exposures do not decrease over a period of several days, there may still be an unknown or undetected source of exposure in the living or working environment. It is also possible that there a sink in the body from where a xenobiotic substance or a product of biotransformation is released into the circulation at a slow rate. This can be a depot of scarcely water-soluble material in the lung lumen, such as deposited ultrafine particles from welding fumes (Scheepers et al, 2008), a sink of a lipophilic substance in adipose tissue, such as in exposures to dioxin (Sorg et al, 2003), a substance that is protein-bound and slowly released due to the long life span of a cell in the case of hemoglobin adducts (Bader and Wrbitzky, 2006), or a tissue depot of protein-bound xenobiotic substance in the kidneys, such as in the case of long-term exposure to cadmium (Sangster et al, 1984).

The use of HBM will be illustrated in a study of dermal absorption of PAH from coal tar. PAH represent a group of substances with a high molecular weight and high lipophilicity. Only judged by their substance properties, dermal absorption would be considered negligible. However, dermal administration of coal-tar in human volunteers demonstrated that benzo[a]pyrene (BaP) is effectively absorbed. As diffusion is the transport process in skin absorption, the concentration gradient over the stratum corneum is the driving force of skin permeation. Metabolism of BaP by CYP1A1 in the dermis increases the concentration gradient and explains the dermal uptake of BaP and other PAH (Fig. 53.2).

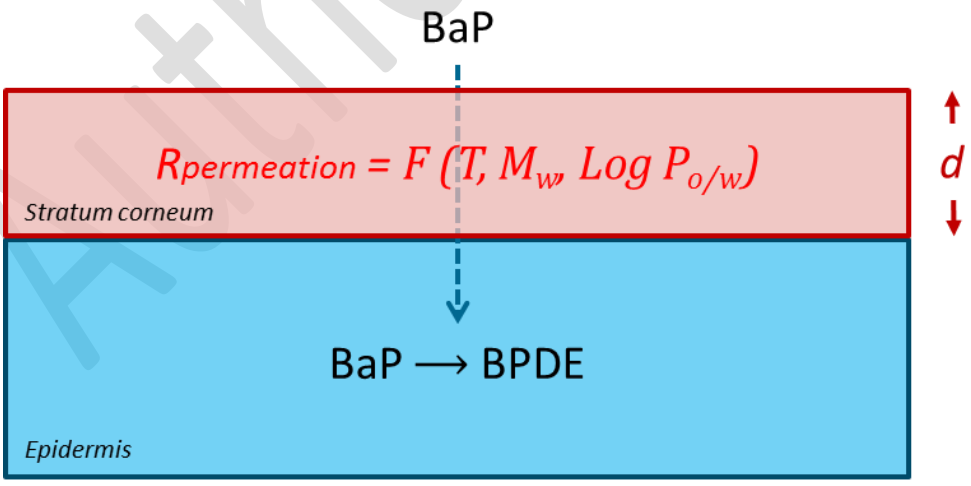

Figure 53.3 Conceptual model for stratum corneum permeation of benzo[a]pyrene (BaP) that represents the most important rate-limiting step to skin absorption of BaP. The aborption is driven by a concentration gradient that is enhanced by biotransformation of BaP to BPDE by Cytochrome P450 1A1 in the epidermis.

The skin absorption of PAH in workers was demonstrated in road paving workers by using 1-hydroxypyrene as a biomarker of exposure and bioactivation (Fig. 53.2). Urinary excretion increased from pre-shift to post-shift urine samples in workers exposed to coal tar-doped asphalt (Jongeneelen et al, 1988). That diffusion is the ruling physical process was also demonstrated in a well-controlled study by Roelofzen and co-workers (2012), comparing uptake of PAH in psoriasis patients who received coal tar therapy with the uptake following a similar treatment in healthy volunteers. The uptake in psoriasis patients was slowed down as reflected in a lower level of total PAH adducts, as well as specific BaP adducts (Fig 53.4 and Table 53.2), and a lower urinary excretion of 1-hydroxypyrene over the entire period of the treatment compared to observed levels in healthy subjects (Fig. 53.5).

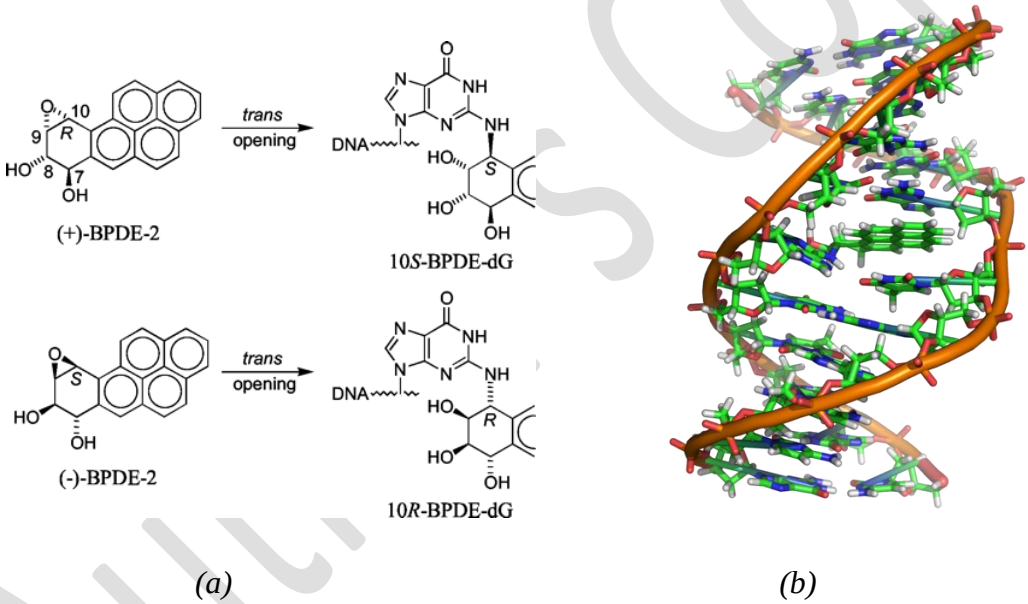

Figure 53.4 (a) Structures of the trans-opened BPDE adducts at N^2 on the guanine base of DNA. A single site-specific trans-opened 7,8,9,10-tetrahydrobenzo[a]pyrene 7,8-diol 9,10-epoxide N^2-deoxyguanosine adduct (from: Kramata et al, (2003). (b): Structure of an adduct of (+)-(7S,8R,9S,10R)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene in a DNA duplex (Wikimedia Commons, the free media repository). The authors suggested that this was a direct result of a smaller distance to be covered in the stratum corneum of healthy subjects compared with patients who have a much thicker stratum corneum as a result of their disease.

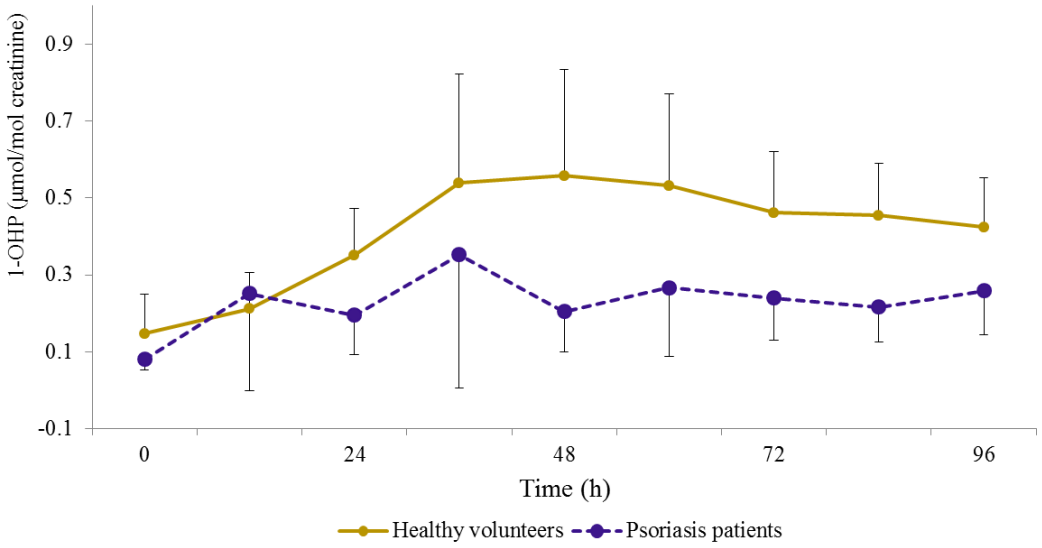

Figure 53.5 Urinary excretion of 1-OHP (error bar indicates standard error of the mean). Time t = 0 h marks the time just before the start of topical application of coal tar ointment. Exposure continued over 96 h. The reduced uptake in psoriasis patients is explained by a disease-related increased thickness of the stratum corneum that contributes to a slower uptake of pyrene. (Source: Roelofzen et al, 2012)

Table 53.3 Median levels (range) of sum of PAH adducts and BPDE DNA adducts in volunteers and patients before and 96 h after the start of application of coal tar ointment (CTO). Adduct levels were measured by ^{32}P-postlabeling and expressed per 108 nucleotides. * $p < 0.05$ compared to baseline (Roelofzen et al., 2012).

Study group	**Before CTO administration (t = 0 h)**		**After CTO administration (t = 96 h)**	
	Sum of PAH-DNA	BPDE-DNA	Sum of PAH-DNA	BPDE-DNA
Healthy subjects (N = 10)	3.5 (1.0-5.2)	< 0.1	21.1 (12.9-29.2)*	8.2 (3.9-13.3)*
Patients (N = 10)	1.0 (0.6-2.9)	< 0.1	3.6 (2.1-18.9)*	1.1 (0.5-6.7)*

53.5.4 Communication strategies

Despite a clear explanation prior to the start of an HBM campaign the meaning of the results of HBM may not be entirely clear. Following an analysis of body tissues, study participants often expect to receive information on their present or future health status. The researchers should explain that most biomarkers reflect uptake, bioavailability, and some also bioactivation. Only a few biomarkers reflect physiological responses that can be interpreted as a predictor of a health-related outcome. Also, these responses will likely disappear because the effects are most often reversible. Only those responses that can be interpreted as adverse in the short-term (e.g., elevated carboxyhemoglobin, inhibition of acetyl cholinesterase activity, elevated methaemoglobin level) may require therapy (see Chapter 54). A continued internal exposure over long to almost life-long exposure may be a risk factor for the occurrence of chronic disease and may also lead to different types of cancer or non-cancer related organ failure. For cancer, many of those exposures have been identified in populations of workers and are listed as human carcinogens by the WHO (see. http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf).

HBM may help to confirm a suspected exposure to a hazardous and potential reproduction toxic and/or carcinogenic risk factor and measures can and should be considered to take away or reduce the sources of such exposures to prevent the occurrence of disease. Only if exposures are extremely high, may the elevated levels of biomarkers explain the occurrence of clinical signs of intoxication that may earlier not have been attributed to a chemical exposure. Such results require immediate and appropriate treatment (see chapter 54). In most cases, biomarker levels will be low and will not explain clinical symptoms. Often exposures are too short (accidents) or there was no direct contact, the direct contact did not lead to a significant uptake or the uptake did not result in a systemic exposure of biological significance. Based on a well-informed interpretation, a physician can often reassure the study subject that health consequences are not likely in the short or long term (Scheepers et al, 2014).

Physicians who are not familiar with the interpretation of laboratory results related to the biomarkers discussed in this section should consult a national or regional poison center for support in interpretation of lab results regarding how to communicate these results to the study subjects, including the consequences of engaging in some kind of treatment to reduce health implications and the potential side-effects of such medical interventions. In many

cases, biomonitoring results will just confirm that supportive medical treatment is sufficient to prevent any adverse health effects resulting from toxic exposures (see Chapter 54). If requested, the results of HBM should be made available and explained to the individual study participant.

53.6 DISCUSSION

Analytical capability, either sensitivity or specificity, augments the potential use of the analysis of body tissues in human health risk assessment. A positive finding of a xenobiotic substance in a body fluid does not represent a risk to health by itself. A positive biomarker finding should be put in perspective by use of contextual information, including both person characteristics and exposure information. This can be done by applying HBM in carefully designed population-based studies where data can become much more valuable if sample collection is repeated in time (Chadeau-Hyam et al, 2013). Temporal patterns of biomarkers can be studied and interpreted by use of mathematical models. In the near future, it is expected that bio-banks will provide valuable resources for the use of HBM approaches in population-based studies. HBM will contribute to studies aimed at describing the totality of environmental exposures from conception onward (Scheepers et al, 2013). These so-called exposome studies are trying to complement the interest in genome characterization by study of the individual's internal and external exposome in connection to temporal shifts in environmental exposures, so-called critical life stages, such as conception, adolescence, pregnancy, and major changes in working environment, such as a new job or in living environment such as migration (Vineis et al, 2013). Biomarkers will help us to understand the role molecular mechanisms in associations between environmental factors and observed responses in human populations (Chadeau-Hyam et al, 2011). These changes result in physiological responses that lead to higher or lower risk in short- and long-term health effects that are of interest to public health policy.

53.7 CONCLUSIONS

The impact of environmental exposures is strongly dependent on the amount of a xenobiotic substance reaching a critical level in one or more internal organs. Analysis of biomarkers in body tissues demonstrates an internal exposure even in persons that do not have the clinical signs of intoxication. Such exposure may originate from different sources and reach the target tissue via different routes of uptake and biotransformation pathways. Biomarkers of susceptibility and effect can demonstrate an early response of human physiology to an exposure that may initially not be detected or evaluated as a health risk. Genes interact with the environment and explain differences in individual responses within a population, even if exposures across the population are quite similar. HBM deserves a prominent position within the field of contemporary public health research.

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