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ScienceDirect
Int. J. Hyg. Environ.-Health 210 (2007) 201–228
International Journal of Hygiene and Environmental Health
www.elsevier.de/ijheh

REVIEW

Human biomonitoring: State of the art

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Abstract

Human biomonitoring (HBM) of dose and biochemical effect nowadays has tremendous utility providing an efficient and cost effective means of measuring human exposure to chemical substances. HBM considers all routes of uptake and all sources which are relevant making it an ideal instrument for risk assessment and risk management. HBM can identify new chemical exposures, trends and changes in exposure, establish distribution 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. The sensitivity of HBM methods moreover enables the elucidation of human metabolism and toxic mechanisms of the pollutants. So, HBM is a tool for scientists as well as for policy makers. Blood and urine are by far the most approved matrices. HBM can be done for most chemical substances which are in the focus of the worldwide discussion of environmental medicine. This especially applies for metals, PAH, phthalates, dioxins, pesticides, as well as for aromatic amines, perfluorinated chemicals, environmental tobacco smoke and volatile organic compounds. Protein adducts, especially Hb-adducts, as surrogates of DNA adducts measuring exposure as well as biochemical effect very specifically and sensitively are a still better means to estimate cancer risk than measuring genotoxic substances and their metabolites in human body fluids. Using very sophisticated but nevertheless routinely applicable analytical procedures Hb-adducts of alkylating agents, aromatic amines and nitro aromatic compounds are determined routinely today. To extend the spectrum of biochemical effect monitoring further methods should be elaborated which put up with cleavage and separation of the adducted protein molecules as a measure of sample preparation. This way all sites of adduction as well as further proteins, like serum albumin could be used for HBM. DNA-adducts indicate the mutagenicity of a chemical substance as well as an elevated cancer risk. DNA-adducts therefore would be ideal parameters for HBM. Though there are very sensitive techniques for DNA adduct monitoring like P32-postlabelling and immunological methods they lack specificity. For elucidating the mechanism of carcinogenesis and for a broad applicability and comparability in epidemiological studies analytical methods must be elaborated which are strictly specific for the chemical structure of the DNA-adduct. Current analytical possibilities however meet their borders. In HBM studies with exposure to genotoxic chemicals especially the measurement of DNA strand breaks in lymphocytes and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in white blood cells has become very popular. However, there is still a lack of well-established dose–response relations between occupational or environmental exposures and the induction of 8-OHdG or formation of strand breaks which limits the applicability of these markers. Most of the biomarkers used in population studies are covered by standard operating procedures (SOPs) as well as by internal and external quality assessment schemes. Therefore, HBM results from the leading laboratories worldwide are analytically reliable and comparable. Newly upcoming substances of environmental relevance like perfluorinated compounds can rapidly be assessed in body fluids because there are very

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1438-4639/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijheh.2007.01.024

powerful laboratories which are able to elaborate the analytical prerequisites in due time. On the other hand, it is getting more and more difficult for the laboratories to keep up with a progress in instrumental analyses. In spite of this it will pay to reach the ultimate summit of HBM because it is the only way to identify and quantify human exposure and risk, elucidate the mechanism of toxic effects and to ultimately decide if measures have to be taken to reduce exposure. Risk assessment and risk management without HBM lead to wrong risk estimates and cause inadequate measures. In some countries like in USA and in Germany, thousands of inhabitants are regularly investigated with respect to their internal exposure to a broad range of environmentally occurring substances. For the evaluation of HBM results the German HBM Commission elaborates reference- and HBM-values.
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Keywords: Human biomonitoring; Protein adducts; Hb-adducts; Biochemical effect monitoring; Comet assay; DNA adducts; Metals; PAH; Phthalates; Dioxins; Pesticides; Aromatic amines; Perfluorinated chemicals; Environmental tobacco smoke; Volatile organic compounds

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Introduction

The determination of chemical substances in human body fluids was first used in occupational medicine for health protection of exposed workers. The determination of lead (Kehoe et al., 1933) or benzene metabolites (Yant et al., 1936) in blood and urine are early examples of human biomonitoring (HBM) of workplace exposures. In the early 1960s, powerful analytical techniques that allowed to measure very low concentrations of chemical substances in blood and urine began to enter the laboratories. These techniques provided the possibility to determine much lower concentrations of chemical substances in human body fluids caused by environmental exposure. Using atomic absorption spectroscopy, e.g., it turned out that the general population of industrialized countries was exposed to lead in a degree that required immediate action. As a consequence, the lead content of gasoline was reduced and for the first time HBM was used in great population studies to determine the blood lead levels and to control the success of the measures taken. In 1977, the Commission of the European Communities (CEC) enacted the ‘‘council directive on biological screening of the general population for lead’’ (Council directive 77/312/EEC, 1977). This directive required that the member states of the European Union should take the necessary steps of applying a common procedure for biological screening in order to assess the exposure of the population to lead outside the work environment. Apart from a directive

on the control of lead exposure at the workplace this was the only HBM activity of the Commission of the European Union for the next 30 years. However, in some countries national authorities started to apply the advantages of HBM in population surveys designed to monitor exposure to environmental pollutants of the general population. The German Environmental Surveys were started in 1985 (GerES I–IV, 1985–2003; Schulz et al., 2007a). The US National Health and Nutrition Examination Surveys (NHANES 1976–2004) regularly determine toxic substances in blood and urine of the general population (Needham et al., 2007). In 2004, the commission of the EU agreed to the European Environment and Health Action Plan where the member states confirmed their interest in "developing a coherent approach to biological monitoring" of the general population. It seems that within the 7th Framework Programme of the EU, HBM shall play a prominent role (Open Stakeholder Consultation, 2006). In the USA, an enthusiastic discussion about the advantages of biomonitoring took place in the last years that even entered public debate (Wanjek, 2004). The Health and Environmental Science Institute (HESI, 2004) of the International Life Science Institute (ILSI) created a Technical Committee on Biomonitoring with the goal to delineate the appropriate scientific use of HBM data and to define the criteria needed for the integration of HBM data into the risk assessment process (Angerer et al., 2006). The US National Research Council was asked by the US Congress to perform an independent study on the possibilities of biomonitoring (NRC, 2006). These activities clearly demonstrate, that HBM is a very actual issue in public health politics, in environmental medicine and in science.

It is the goal of this article to summarize the current state of the art of HBM mainly focussing on monitoring of exposure, and of biochemical effect. Additionally, the usefulness of DNA damage as measured by the comet assay in HBM studies will be discussed. The usefulness and limitations of other biological effect markers of genotoxicity such as sister chromatid exchanges, micronuclei and chromosome aberrations are described in another paper of this issue (Au, 2007).

Human biomonitoring (HBM)

HBM is a method out of two for the protection of human health in case of exposure to chemical substances by controlling the amounts taken up. In 1980, during the period of EU activities in HBM Zielhuis (1984) suggested the following definition for HBM: HBM is "a systematic continuous or repetitive activity for collection of biological samples for analysis of concentrations of pollutants, metabolites or specific non-adverse biological effect parameters for immediate application, with the objective to assess exposure and health risk to exposed subjects, comparing the data observed with the reference level and — if necessary — leading to corrective actions". HBM today differentiates between dose monitoring, biochemical effect monitoring and biological effect monitoring (Angerer and Gündel, 1996; Kommission HBM, 1996a). Dose monitoring is the determination of hazardous substances or their metabolites in body fluids. Biochemical effect monitoring is the quantification of the reaction products of reactive substances with biological molecules such as DNA or proteins. Biological effect monitoring is related to the measurement of early biological effects caused by chemical substances, for instance sister chromatid exchange rates, micronuclei, enzyme activities (Fig. 1).

The other method to protect human health in case of exposure to chemical substances is called ambient monitoring (AM) which means determination of chemical substances in environmental matrices like air, water soil, food, etc. HBM is considered to supplement AM and not to completely replace it. AM is especially necessary to identify the sources of exposure and to facilitate measures for minimizing emissions.

For the estimation of the dose really taken up and for its risk to human health HBM is indispensable because HBM shows whether and to what extent chemical substances are really taken up from the environment (internal dose). This is very important basic information which cannot be supplied by AM of air, water, house dust, etc. HBM can assess the dose really taken up without using worst case scenarios which regularly lead to an overestimation of exposure (Angerer et al., 2004; Suchenwirth et al., 1996).

In Germany for instance billions of Euro were spent for decontamination of buildings where PCB have been used as plasticizer for sealants. HBM showed that PCB uptake in such buildings were two to three orders of magnitude lower than calculated (Liebl et al., 2004). That HBM can identify new chemical exposure of the general population has been shown since the early 70s of the last century when internal lead exposure of the population reached hematotoxic concentrations. The most current example is the occurrence of perfluorinated chemicals, which can be found in every blood sample of the population (Table 1) (Midasch et al., 2006; Fromme et al., 2007; Calafat et al., 2006a, b). Using HBM trends and changes in exposure can be observed. The efficacy of measures like the reduction of lead in gasoline or the ban of organochlorine compounds like DDT or PCB led to a dramatic decrease of internal exposure of the general population (e.g., Schulz et al., 2007a; Wilhelm et al., 2007; Wittsiepe et al., 2000, 2007). HBM moreover enables to identify groups of the population which are at higher risk. Children for instance have higher uptakes of pesticides like organophosphates or pyrethroids with

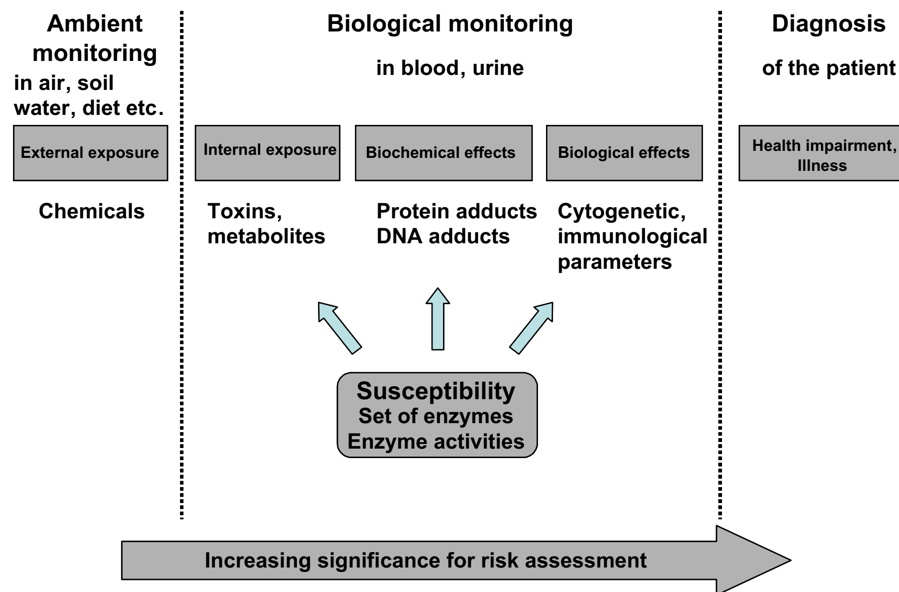

Fig. 1. Scheme of ambient and biological monitoring (modified acc. DFG, 2002; Angerer and Gündel, 1996).

Table 1. Biomarkers of internal exposure in environmental medicine (blood, urine)

(a) Metals

Metals	Matrix blood (b), urine (u)	References
As; (Ba); Be; Cd; Co	u	CDC, 2005; GerES I–IV, 1985–2003
Cr; (Cs); (Cu); Hg; Mo; Pb		CDC, 2005; GerES I–IV, 1985–2003
Pt; Sb; (Se); Tl; V; U; (Zn)		CDC, 2005; GerES I–IV, 1985–2003
Ni	u	Wilhelm et al., 2004
Cd; (Cu); Hg; Pb	b	CDC, 2005; GerES I–IV, 1985–2003
(Se); (Zn)		

(b) Polyaromatic hydrocarbons (PAH)

Metabolites	Matrix	References
1-,3-,9-Hydroxybenz[*a*]anthracene	u	CDC, 2005; GerES I–IV, 1985–2003
1-,2-,3-Hydroxybenzo[*c*]phenanthrene	u	CDC, 2005; GerES I–IV, 1985–2003
1-,2-,3-,4-,6-Hydroxychrysene	u	CDC, 2005; GerES I–IV, 1985–2003
3-Hydroxyfluoranthene	u	CDC, 2005; GerES I–IV, 1985–2003
2-,3-,9-Hydroxyfluorene	u	CDC, 2005; GerES I–IV, 1985–2003
1-,2-,3-,4-,9-Hydroxyphenanthrene	u	CDC, 2005; GerES I–IV, 1985–2003
1-Hydroxypyrene	u	CDC, 2005; GerES I–IV, 1985–2003
3-Hydroxybenzo[*a*]pyrene	u	CDC, 2005; GerES I–IV, 1985–2003
1-,2-Hydroxynapthalene	u	CDC, 2005; GerES I–IV, 1985–2003

(c) Polychlorinated dibenzodioxins (PCDD), polychlorinated biphenyls (PCB)

Substances	Matrix	References
1,2,3,4,6,7,8,9-Octachlorodibenzo-*p*-dioxin	b	CDC, 2005
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	b	CDC, 2005
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	b	CDC, 2005
1,2,3,4,6,7,8-Heptachlorodibenzofurane	b	CDC, 2005
Dioxin-like PCB		
PCB (126;169)	b	CDC, 2005
Non-dioxin-like PCB		

Table 1. (*continued*)

(c) Polychlorinated dibenzodioxins (PCDD), polychlorinated biphenyls (PCB)

Substances	Matrix	References
2,2′,3,4,4′,5′-Hexachlorobiphenyl (PCB 138)	b	CDC, 2005
2,2′,4,4′,5,5′-Hexachlorobiphenyl (PCB 153)	b	CDC, 2005
2,2′,3,3′,4,4′,5-Heptachlorobiphenyl (PCB 170)	b	CDC, 2005
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB 180)	b	CDC, 2005

(d) Phthalates

Metabolite	Matrix	References
Mono-methyl-phthalate	u	CDC, 2005
Mono-ethyl-phthalate	u	CDC, 2005
Mono-*n*-butyl-phthalate	u	CDC, 2005
Mono-isobutyl-phthalate	u	CDC, 2005
Mono-benzyl-phthalate	u	CDC, 2005
Mono-cyclohexyl-phthalate	u	CDC, 2005
Mono-*n*-octyl-phthalate	u	CDC, 2005
Mono-(3-carboxypropyl)phthalate	u	CDC, 2005
Monoethylhexylphthalate (MEHP)	u	CDC, 2005
Mono-(2-ethyl-5-hydroxy-hexyl)phthalate (5-OH-MEHP)	u	CDC, 2005
Mono-(2-ethyl-5-oxohexyl)phthalate (5-oxo-MEHP)	u	CDC, 2005
Mono-(2-ethyl-5-carboxypentyl)phthalate (5-cx-MEPP)	u	Koch et al., 2005
Mono-(2-(carboxy-methylhexyl)phthalate (2-cx-MMHP)	u	Koch et al., 2005

(e) Pesticides

Metabolite	Matrix	References
Organophosphate pesticides		
Dimethylphosphate (DMP)	u	GerES I–IV, 1985–2003
Dimethylthiophosphate (DMTP)	u	GerES I–IV, 1985–2003
Dimethyldithiophosphate (DMDTP)	u	GerES I–IV, 1985–2003
Diethylphosphate (DEP)	u	GerES I–IV, 1985–2003
Diethylthiophosphate (DETP)	u	GerES I–IV, 1985–2003
Diethyldithiophosphate (DEDTP)	u	GerES I–IV, 1985–2003
***p*-Nitrophenol**	u	CDC, 2005
3,5,6-Trichloro-2-pyridinol	u	CDC, 2005

Substances, metabolites	Matrix	References
Organochlorine compounds		
Hexachlorobenzene (HCB)	b	GerES I–IV, 1985–2003
Hexachlorocyclohexane (α,β,γ HCH)	b	CDC, 2005
1,1′-(2,2-dichloroethenylidene-bis(4-chlorobenzene) (DDE)	b	CDC, 2005
Chlordane (oxychlordane, *t*-nonachlor)	b	CDC, 2005
Heptachlorepoxid	b	CDC, 2005
Mirex	b	CDC, 2005
Aldrin	b	CDC, 2005
Dieldrin	b	CDC, 2005
Endrin	b	CDC, 2005
4-Monochlorophenol	u	CDC, 2005
2,4-Dichlorophenol	u	CDC, 2005

Table 1. (*continued*)

Substances, metabolites	Matrix	References
2,5-Dichlorophenol	u	CDC, 2005
2,4,5-Trichlorophenol	u	CDC, 2005
2,4,6-Trichlorophenol	u	CDC, 2005
2,3,4,6-Tetrachlorophenol	u	CDC, 2005
Pentachlorophenol	u	CDC, 2005

Metabolites	Matrix	References
Pyrethroids		
3-Phenoxybenzoic acid (3-PBA)	u	GerES I–IV, 1985–2003; CDC, 2005
***cis*- and *trans*-(3-2,2 dichlorvinyl)-2,2 dimethylcyclopropane-1-carboxylic acid (*cis*-*trans*-DCCA)**	u	GerES I–IV, 1985–2003; CDC, 2005
4-Fluoro-3-phenoxybenzolic acid (F-PBA)	u	GerES I–IV, 1985–2003; CDC, 2005
3-(2,2-Dibromovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (DBCA)	u	GerES I–IV, 1985–2003; CDC, 2005
Other pesticides		
***o*-Phenylphenol**		CDC, 2005

(f) Aromatic amines

Substances, metabolites	Matrix	References
Aniline	u	Weiß et al., 2000
***o*-Toluidine**	u	Weiß et al., 2000
***m*-Toluidine**	u	Weiß et al., 2000
***p*-Toluidine**	u	Weiß et al., 2000
3,5-Dichloroaniline	u	Weiß et al., 2000
***o*-Anisidine**	u	Weiß et al., 2000
4-Chloroaniline	u	Weiß et al., 2000
2-Aminonaphthalene	u	Riedel et al., 2006
4-Aminobiphenyl	u	Riedel et al., 2006

(g) Perfluorinated substances

Metabolites	Matrix	References
Perfluorooctane sulfonate (PFOS)	b	Olsen et al., 2003, 2004
Perfluorooctanoate (PFOA)	b	Midasch et al., 2006
		Fromme et al., 2007
		Calafat et al., 2006a, b
		Kärrman, 2004
		Kannan et al., 2004
		Falandysz et al., 2006
		Harada et al., 2004

(h) Tobacco smoke

Metabolites	Matrix	References
Nicotine, cotinine	b, u	CDC, 2005 GerES I–IV, 1985–2003

Table 1. (*continued*)

(i) Volatile organic compounds (VOC)

Substances	Matrix	References
Aromatic-, halogenated-hydroxycarbons	b	Brugnone et al., 1989 Angerer et al., 1991 Ashley et al., 1992 Ashley et al., 1994 Sexton et al., 2006

Metabolites of VOC

Substance, metabolites	Metabolites	Matrix	Reference
Benzene	*t*-,*t*-Muconic acid	u	Eikmann et al., 2000
	S-phenylmercapturic acid	u	
Styrene, ethylbenzene	Mandelic acid	u	Manini et al., 2002; Ohashi et al., 2006
Phenoxyethanol	Phenylglyoxylic acid	u	Ben-Brik et al., 2004
Methoxypropanol	Phenoxy acetic acid	u	
	Methoxy propionic acid	u	

Bold letters: marker found in more than 50% of the samples.
Normal letters: marker found in less than 50% of the samples.

diet than adults (Becker et al., 2006). In the case of phthalates children not only have a higher uptake, they also oxidize a higher amount of the monoesters to form toxic metabolites (Koch et al., 2006). That means that the sensitivity of modern HBM methods enables to elucidate human metabolism in vivo and without experimental exposure, this way adding much to the elucidation of toxic mechanism of a special pollutant. Of course, HBM can be used highly effectively if specific contaminated sites like land fills, waste incinerators, power plants, industrial settings, etc. which are supposed to cause additional exposure to hazardous chemical substances. In all these cases HBM results not only reflect exposure better than measuring chemicals in air, water, soil, house dust, etc., but it is moreover much cheaper than ambient monitoring. It is the great advantage of HBM that it considers all routes of uptake—oral, dermal, inhalative—and all sources which are relevant for human uptake. Therefore, HBM has a tremendous utility for exposure and risk assessment, for health prevention, for risk management and for policy making. Last but not least it has to be pointed out that HBM can within population studies contribute much to the elucidation of toxic mechanisms, metabolism and effects of chemical compounds. The capability of HBM has recently been evaluated and summarized by national and international bodies like the National Academy of Sciences of the USA (2006), the Health and Environmental Science Institute (HESI, 2004) of the International Life Science Institute and the Deutsche Forschungsgemeinschaft (DFG, 2002).

Requirements for HBM

HBM requires the following to be fulfilled:

(a) suitable biological matrices,
(b) suitable parameters, able to reflect internal exposure, biochemical or biological effects,
(c) suitable and reliable analytical methods which are kept under control by quality assurance, and
(d) reference and limit values which enable the interpretation of results.

Biological matrices

Biological materials should easily be accessible in sufficient amounts under routine conditions and without unacceptable discomfort and health risk for the individual. For these reasons blood and urine are the biological matrices most commonly used. Blood as the central compartment is in steady state with all organs. Urine is still more readily and in large volumes accessible allowing the determination of very low

concentrations of chemicals caused by environmental exposure.

Hair, pulmonary air, teeth, nails and saliva also have been used for HBM purposes. Application of these matrices is hampered due to many reasons. For all of these matrices no standard operating procedures (SOPs) have been evaluated and published until now. External quality assessment schemes for these materials are not available. Furthermore, there are no reliable reference values or limit values for the interpretation of the results. Each of these matrices has a couple of further shortcomings. In the case of hair, exogenous contamination, the kind of hair care, etc. influences the analytical results (Wilhelm and Idel, 1996; Kommission, 2005). Gulson found that the lead concentration in nails obviously has a very high intra-individual variation so that this matrix seems to be not suitable for HBM (Gulson, 1996). However, for special purposes hair and nails serve as an appropriate matrix for exposure assessment in environmental epidemiology, e.g., to assess methyl mercury intake via fish consumption (van Wijngaarden et al., 2006) or exposure to inorganic arsenic (Karagas et al., 2002; Wilhelm et al., 2005). Sampling, transportation and storage of pulmonary air samples are critical due to losses, e.g. by absorption. Such practical problems impeded a broad application of this matrix. Teeth are not appropriate for biomonitoring because they are not readily available. Timchalk et al. (2004) showed that saliva can be used as a biological matrix in the case of lead and organophosphate exposure, while Wilhelm et al. (2002) could not confirm the suitability of saliva for lead exposure assessment. However, until now saliva does not fulfil the prerequisites of HBM to be applied in individual measurements and in population studies.

Biomarkers

Biomarkers of exposure

Which biomarkers can nowadays be determined in human body fluids to estimate the dose taken up? In Table 1 those biomarkers are depicted which have shown their diagnostic reliability and applicability in great population studies. Most of these markers indicate exposures to environmental pollutants which are important for public health. Therefore, these markers were used in large population surveys such as the German Environmental Surveys (GerES I–IV, 1985–2003) (Becker et al., 2002, 2003) and the US national health and nutrition examination survey (NHANES, 1999–2000). These surveys are protruding with respect to the number of participants—several thousands each—and the number of parameters that were determined. It should be pointed out that in all these surveys representative groups of the general population were studied. Therefore, the HBM data are representative for the whole population of Germany and the United States, respectively. Table 1 also contains biomarkers of exposure which could and should be determined in further HBM studies, because those markers have shown their importance for public health. This applies for instance to perfluorinated surfactants such as PFOS and PFOA (Midasch et al., 2006; Fromme et al., 2007; Calafat et al., 2006a, b) and also to aromatic amines (Weiß et al., 2000; Weiß, 2005). According to recent studies these compounds can be determined in plasma samples of the whole population. All the parameters depicted in Table 1 must be considered relevant for public health. Substances printed in bold letters are found in blood or urine of more than 50% of the population. Substances printed in normal letters are found in blood or urine samples of less than 50% of the population. Detailed descriptions about the use, toxicological profiles, uptake, metabolism of the environmental pollutants which underlie Table 1 are available from several national and international bodies (IARC, 1971–2007; IPCS, 1980–2007; ECETOC, 1978–2007; ATSDR; NTP, a, b; DFG, 1972–2006; BUA, 1986–2007; etc.).

Metals

Out of the depicted metals (Table 1) especially arsenic, cadmium, mercury, lead and to a minor extent nickel, antimony, thallium, and platinum are of health concern for the general population. Arsenic, cadmium, lead, and nickel are carcinogenic to humans or animals. Arsenic is released into the environment by natural and industrial activities: the combustion of coal and industrial processes like iron, copper, and lead smelters are considered as the main sources of arsenic emission. By the general population arsenic is taken up mainly with fish. In this case various arsenic species like inorganic and organic compounds are taken up. Cadmium enters the environment primarily by anthropogenic activities like lead production, municipal waste incineration, petroleum refining, etc. Lead exposure due to leaded gasoline has dramatically been reduced in the last years. Occupational and recreational activities nowadays may lead to an increased uptake of lead. Lead water pipes, lead based paint, lead glazed ceramic pottery, home burning, etc. are further possible sources of lead uptake. Nickel and its compounds are also mainly taken up with diet and are suspected to enhance nickel induced allergies. However, nickel orally taken up is not carcinogenic. Elemental mercury is released into the air from the combustion of fossil fuel, waste incineration, mining and smelting. Water can be contaminated by the direct release of mercury from industrial processes. Microorganisms in water transform inorganic mercury into organomercury compounds which bioaccumulate in the aquatic food chain. The ingestion of methyl mercury predominantly from fish constitutes the

main source of mercury exposure in the general population. Antimony enters the environment from natural sources and from its use in industry. Environmental exposure to thallium primarily results from industrial processes like coal-burning and smelting. The main source of platinum uptake are dental alloys (Philippeit et al., 2000; Herr et al., 2003).

PAH

Polyaromatic hydrocarbons (PAH) (Table 1(b)) are products of incomplete combustion of organic materials, which can be found ubiquitously in the environment. Some PAH are potent carcinogenics and complex mixtures of PAH have been shown to be carcinogenic to humans. Although the PAH levels in ambient air were reduced significantly in the last 25 years, PAH are still relevant in environmental medicine. HBM of exposure to PAH is based on the measurement of some PAH metabolites in urine, which turned out to be reliable and specific: 1-hydroxypyren, hydroxyphenanthrenes and 1,2-hydroxynaphthalene. The determination of 3-hydroxybenzo[*a*]pyrene in urine is a great progress because this substance has a high carcinogenic potency and is therefore used as an indicator for the whole spectrum of PAH in ambient monitoring. However, it is not clear if this parameter is suitable for routine application in environmental medicine. This is also true for hydroxyfluoranthene and hydroxyfluorene.

Phthalates

Phthalates (Table 1(d)) are a group of substances which differ with respect to the length of their aliphatic side chain. They are used for a lot of different purposes like paints, cosmetics, etc. The most important application of phthalates however is the use as plasticizers mainly for PVC. Millions of tons are annually used for this purpose. Phthalates leak out of these material and are ubiquitously distributed in the environment. In the last few years it turned out that the general population takes up much more phthalates than expected (BfR, 2003; UBA, 2006; Koch et al., 2003). The most important source of uptake seems to be the diet. This is of great concern because phthalates are endocrine disrupting chemicals. In animal studies it was shown that exposure to phthalates is associated with decreased testosterone levels, testicular atrophy, sertoli cell abnormalities and other effects on the reproductive system (CERHR, 2000; CSTEE, 2004; Hauser and Calafat, 2005). Most recent results show that phthalate exposure may be associated with a change of several parameters and a reduction of the anogenital distance (Duty et al., 2003a, b, 2004, 2005; Swan et al., 2005).

PCDD, PCDF, PCB

Polychlorinated dibenzodioxins (PCDD), polychlorinated dibenzofuranes (PCDF) and polychlorinated biphenyls (PCB) (Table 1(c)) are persistent organic pollutants which, due to their immunotoxic and neurotoxic properties, are still a matter of concern. They are taken up mainly with diet and from various environmental sources especially incineration processes. Using very sophisticated analytical methods PCDD, PCDF, and PCB can be determined in blood or plasma samples in highly specialized laboratories. The analytical requirements for the determination of the other PCB are more moderate. Except of some PCB (Wilhelm et al., 2004) for Germany no representative data are available for most PCDD/F and other PCB. However, more than 1000 blood samples have been analysed for PCDD/F since the late 1980s in North Rhine Westphalia giving a good estimate of the background exposure of the general German population (Wittsiepe et al., 2000, 2007; Wilhelm et al., 2007).

Pesticides

Pesticides (Table 1(e)) like insecticides, herbicides, etc. are used all over the world mainly for crop protection. Therefore, dietary uptake is considered the main source of exposure in humans. Insecticides are also used for livestock, pets and indoor pest control.

Organophosphate (OP) insecticides are triesters of phosphorus-, thiophosphorus- and dithiophosphorus-acid. They are used in agriculture since the 19th century. In the US 36 OPs are registered for use (Barr and Angerer, 2006). OPs are well known neurotoxins which inhibit the cleavage of the neurotransmitter acetylcholine. In the human body OPs are hydrolysed yielding six dialkylphosphates which are excreted in urine. These six diesters are used as parameters of internal exposure to all organophosphates used worldwide. This approach has first been used by Shafik and Enos (1969) more than 30 years ago. In the mean-time HBM parameters emerged which measure internal exposure to specific OPs. *p*-Nitrophenol and trichloropyridinol which are found in every urine sample of the population are the metabolites of methyl-parathion, parathion, chlorpyrifos and chlorpyrifos-methyl (Heudorf and Angerer, 2001; CDC, 2005).

Pyrethroids are more modern insecticides which are more neurotoxic to insects and less neurotoxic to humans than organophosphates. This led to lower amounts which have to be applied and to lower exposure of the general population. The most important pyrethroids permethrin, cyfluthrin, cypermethrin and deltamethrin are immediately hydrolysed in the human body. The metabolites 3-PBA, DCCA, F-PBA and DBCA (Table 1) which are excreted in urine are commonly used as HBM parameters (Heudorf and Angerer, 2001; Leng et al., 2003; CDC, 2005).

o-Phenylphenol (OPP) and its sodium salt are used in agriculture as an antimicrobial pesticide. The sodium salt is supposed to be carcinogenic. OPP can be detected

in practically every urine sample collected from individuals of the population.

Many of the organochlorine compounds (OC) which have been used as pesticides like DDT, chlordane, heptachlor, mirex, aldrin, dieldrin, endrin and pentachlorophenol are restricted in use or prohibited in western industrialized countries. They are not degraded in the mammalian organism, and, therefore, they accumulate in the food chain. Due to their lipophilic properties OC are taken up mainly by fatty foods. Breast milk is the major source of OC exposure of infants. There are regions in the world where some of them are still in use for pest control like for instance DDT for malaria prevention. OC enter the environment by production or direct application. Disposal, contaminated waste in land fills, incinerators, power plants or other combustions processes in the presence of chlorine are further sources of OC emission.

Chlorophenols may origin from a broad variety of OC like hexachlorocyclohexene, chlorobenzenes, chlorophenols, chlorophenoxycarbonic acids and herbicides. The urinary excretion of chlorophenols seems to be a good indicator of the background exposure of the general population to various OC from all possible sources (Wrbitzky et al., 1994, 1995). Chlorophenols within the German environmental survey are used as an own group of parameters indicating OC exposure (Becker et al., 2003).

Aromatic amines

In the last years it turned out that a broad spectrum of aromatic amines (AA) (Table 1(f)) could be detected in urine samples of the general population (Teass et al., 1993; Riffelmann et al., 1995; Weiß et al., 2000; Riedel et al., 2006). These results could newly be approved in a study covering more than 1000 persons of the general population in Bavaria, Germany. The observation that the general population is obviously exposed to a broad range of AA must raise concern because most of these substances are confirmed or suspected human carcinogens. In Germany, 5% of cancer diseases is caused by those of the bladder, the target of AA. A lot of sources contribute to the AA excretion of the population. 3- and 4-chloroanilines as well as 3,4- and 3,5-dichloroaniline are metabolites of pesticides which are taken up with diet. The main sources for aniline and *o*-, *m*-, *p*-toluidine are not known till now. Amongst others they have been detected in coffee and tea and they are metabolites of some pesticides like prophame, desmediphame and phenmediphame. The excretion of these AA is also influenced by tobacco smoke, but other sources seem to be much more important. The same is true for *o*-anisidine and 2-naphtylamine whereas 4-aminobiphenyl is a classical marker of tobacco smoking. Keeping the carcinogenic properties of AA in mind these biomarkers should be used hence forward in population studies to estimate possible health risk caused by the background exposure of the general population to these substances.

Perfluorinated substances

Perfluorooctanesulfonate (PFOS) and perfluorooctonate (PFOA) are raw materials for various perfluorinated chemicals (PFCs) (Table 1(g)) that are used in a great variety of products. Major application includes surfactants, surface protection, paper and food package treatment, lubricants, etc. PFOS and PFOA can also be realized from several PFCs by biotic and/or metabolic decomposition (OECD, 2002). Due to their widespread use and their long elimination half-lives PFOS and PFOA can be detected in serum of non occupationally exposed people around the world (Calafat et al., 2006a, b; Falandysz et al., 2006; Harada et al., 2004; Kannan et al., 2004; Kärrman, 2004; Olsen et al., 2003, 2004). The toxicological profiles of PFOS and PFOA in respect to human health effects have been reviewed (OECD, 2002; Butenhoff et al., 2004a; US EPA, 2005) in particular the developmental toxicity in humans is in the focus of recent studies (Butenhoff et al., 2004b; Lau et al., 2003, 2004, 2006; Luebker et al., 2005a, b; Thibodeaux et al., 2003). The concentration of PFOS and PFOA in plasma samples of the general population seems to be a reliable parameter of internal exposure to these substances. PFCs which only recently have been detected in human body fluids are examples for the efficiency of HBM in identifying new chemical exposures of the population. (Table 1(h)).

ETS

Not only tobacco smoking but also exposure to environmental tobacco smoke (ETS) gives raise to great concern. Amongst others ETS is associated with lung cancer and coronary heart disease. ETS may also cause asthma, bronchitis, pneumonia especially in young children (CDC, 2005). Though nicotine is but one of hundreds of chemical substances in ETS, nicotine and its metabolite cotinine are good indicators for internal ETS exposure. Cotinine is a reliable parameter and is more widely used in environmental medicine. Urine or plasma are used as matrices for the estimation of internal exposure (Becker et al., 2003; CDC, 2005).

VOC

Volatile organic compounds (VOC) (Table 1(i)) especially aromatic, halogenated and aliphatic hydrocarbons are ubiquitously occurring in the environment as well as in indoor air. They are constituents of solvents and other consumer products or of traffic exhaust. Benzene which is carcinogenic in humans poses a serious problem in environmental medicine. VOC lead to many unspecific complaints like headache, fatigue, etc. especially in newly renovated residences. Using head space

analysis all these volatile compounds can be determined very sensitively and diagnostically specific preferably in blood samples (Brugnone et al., 1989; Angerer et al., 1991; Ashley et al., 1992, 1994; Sexton et al., 2006).

The excretion of metabolites of VOC in urine offer the advantage that their half-lifes in the human body are longer than that of the solvents themselves. Up till now *S*-phenylmercapturic-, *t*,*t*-muconic and mandelic- and phenylglyoxylic acid, the metabolites of benzene, styrene and ethylbenzene have shown their suitability for biomonitoring (Eikmann et al., 2000; Ohashi et al., 2006). Glycol ethers which are constituents of a lot of consumer products like water based varnishes are oxidized in the human body to yield carboxylic acids like phenoxic acetic and methoxypropionic acid (Ben-Brik et al., 2004). These acids are more toxic than the glycolethers themselves and are excreted with unusually long half-lifes. So these glycol ether metabolites are very good parameters for biomonitoring. Indoor exposure to glycolethers is still a matter of concern. The more toxic glycolethers adversely effect reproduction.

So for practically all environmental pollutants, which are in the focus of the worldwide discussion of environmental medicine, internal exposure of the total population can be measured. This gives raise to apply HBM within great epidemiological studies to correlate exposure to a given substance with resulting health effects. Up to now it has been a decisive shortcoming of population studies that exposure assessment was not appropriate due to data based on environmental monitoring procedures like air, water, oil analysis, etc. or even on questionnaires.

Biomarkers of biochemical effect

Protein adducts—haemoglobin adducts (Hb-adducts)

Mutagenic and carcinogenic substances bind to macromolecules, especially to proteins. That reactive electrophilic intermediates of mutagenic substances bind to nucleophilic sites of proteins is the underlying principle. The preferred sites are the sulfhydryl group of cystein and nitrogen of histidin and N-terminal valine because the pK_a-values are in the range of the pH of blood ($p = 7.4$). Haemoglobin (Hb) and serum albumin (SA) are the preferred monitor molecules because they are accessible in large amounts. They are chemically stable and are not prone to repair mechanisms like DNA-adducts. Because of the long life span of Hb (120 days) and the long half-life of SA (20 days) these adducts cumulate in the human body, making them a very sensitive parameter for HBM.

Hb-adducts are the preferably monitored molecules because cumulation is still greater than that of SA. Moreover, reactive intermediates have to cross a cell membrane showing this way that they are sufficiently stable to reach the DNA in the critical organ. Because of these reasons the scientific community takes Hb-adducts as surrogates of DNA-adducts, which are thought to be the initial step of carcinogenicity. Hb-adduct levels in blood enable the estimation of internal exposure as well as biochemical effects. Hb-adducts seem to be better estimates for cancer risk than measuring the genotoxic substances or their metabolites in human body fluids (DFG, 2002).

Also for practical reasons Hb-adducts are preferred, because the electrophilic intermediates bound to the N-terminal valine can elegantly be cleaved off by an Edmann degradation and can relatively easily be quantified by GC-MS (Törnqvist, 1986; Mowrer et al., 1986). The same is true for aromatic amines and aromatic nitro compounds which bind to sulfhydryl groups of Hb. These substances can hydrolytically be cleaved off from Hb and can also be determined using GC-MS (Lewalter et al., 1985; Stillwell et al., 1987; Bryant et al., 1988; Weiß, 2005). So nowadays in advanced laboratories it is possible to routinely determine the adducts of alkylating substances, aromatic amines and aromatic nitro carbons which are depicted in Table 2.

The ubiquitous occurrence of ethane (E) and ethylene oxide (EO) in the environment is due to industrial processes. As a plant hormone E moreover is released in huge quantities into the environment. In the human body E is oxidized to yield EO that binds amongst others to N-terminal valine. Butadiene like E is a ubiquitously occurring pollutant of the atmosphere which binds to Hb after enzymatic oxidation. Acrylonitril as well as acrylamide (AA) directly bind to Hb in a Michael addition reaction without prior oxidation. In parallel AA, however, is also oxidized to form glycidamide (GA) which also reacts with Hb. GA is the ultimate carcinogenic agent of AA (DFG, 1972–2006). Acrylonitril (ACN) and AA are constituents of tobacco smoke. It has been shown that AA is formed during heating procedures of carbohydrate and protein containing food (Tareke et al., 2002) and that the general population take up carcinogeneous AA with diet. Risk assessment of dietary AA uptake (WHO, 1996; US EPA, 1990) show that AA is a prior environmental carcinogen which need further action.

As already described aromatic amines enter the environment from a lot of difference sources like tobacco smoke, pesticides, food stuff, certain drugs, industrial pollution, etc.

Nitroaromatic compounds which enter the environment from military wastes or as combustion products are reduced to amino-compounds in the human body. The amino group then gives raise to the formation of Hb-adducts which can be determined as already described (Zwirner-Baier and Neumann, 1999; Ewers et al., 2000).

So for some groups of the environmentally most import carcinogens Hb-adducts can routinely be used in population studies. For other genotoxic substances like

Table 2. Markers of biochemical effect. Haemoglobin adducts of mutagenic environmental chemicals (modified according to Human Biomonitoring Commission, Germany 2003)

Chemical	Adduct	Reference
Alkylating agents		
Ethylene, -oxide	Hydroxyethylvaline	Bailey et al., 1988; Angerer et al., 1998
Butadiene, -oxide	*N*-(2-Hydroxy-3-butenyl)valine	Begemann et al., 2001
Acrylonitrile	Cyanoethylvaline	Osterman-Golkar et al., 1994
Acrylamide	*N*-2-Carbamoylethylvaline	Bergmark, 1997; Schettgen et al., 2002
Glycidamide	*N*-(*R*,*S*)-2-hydroxy-Carbamoylethylvaline	Schettgen et al., 2004
Aromatic amine		
Aniline		Lewalter and Korallus, 1985
o-, *m*-, *p*-Toluidine		Bryant et al., 1988; Weiß, 2005
o-Anisidine		Weiß, 2005
2-Aminonaphthalene		Weiß, 2005
4-Aminobiphenyl		Bryant et al., 1987; Weiß, 2005
Nitro aromatic compounds		
2,6-Dinitrotoluene	2-Amino-6-nitrotoluene	Ewers et al., 2000; Hagmann et al., 2004
2,4,6-Trinitrotoluene	2-Amino-4,6-dinitrotoluene	Ewers et al., 2000; Hagmann et al., 2004
1-Nitropyrene	1-Aminopyrene	Zwirner-Baier and Neumann, 1999

PAH, tobacco specific nitrosamines, anhydrides of organic acids, isocyanates, etc. the determination of Hb-adducts have been described (for a review see Törnqvist et al., 2002). These possibilities, however, have not been used in environmental HBM till now. This also applies for adducts at other binding sites than N-terminal valine. In these cases the protein chain has to be cleaved to yield these adducts. Such procedures lead to multiple products, which have to be separated in tedious chromatographic procedures. In spite of all obstacles, however, protein adduct monitoring should be pushed to get a better means of risk estimation of carcinogenic substances.

DNA adducts

The formation of a DNA adduct is the initial step of carcinogenesis (DFG, 2002). DNA adducts are markers of exposure to carcinogenic substances showing carcinogen intake and metabolic activation by forming an ultimate carcinogen which binds to DNA. DNA adducts lend plausibility to an epidemiological association between a chemical substance and cancer. To give an example: DNA adducts in cervical epithelium had been the missing link between tobacco smoking and cervical cancer. Up to now DNA adducts do not allow a quantitative estimate of cancer risk but the occurrence of DNA adducts at least show an elevated cancer risk. Based on these considerations it is stipulated that the potential of DNA adducts should be exploited in epidemiological studies to differentiate between confounders and real risk factors (Phillips, 2005).

Environmentally caused adduct levels are in an order between $1/10^7$ and $1/10^{11}$ nucleosides. To determine these adduct concentrations (a) immunoassays, (b) ^{32}P-postlabelling, (c) HPLC ECD and FD and (d) GC/MS; LC-MS/MS have been used.

For the determination of adducted nucleosides mostly white blood cells (WBCs) and lymphocytes have been used as surrogate tissues. Sputum and exfoliated urothelial cells have been used sporadically to gain DNA. For HBM purposes it is a crucial point to get sufficient DNA from easily accessible human body fluids. In urine preferably adducted DNA bases are determined. They are excreted in urine as a result of enzymatic depurination of DNA sites bearing adducts. The advantage of urinary adducts is the availability of large amounts of urine and that they represent an integrated measure of whole body dose of ultimate carcinogens (Shuker and Farmer, 1992). Disadvantages of urinary adduct levels are that the origin of adducts is unknown whether it is DNA, RNA, free nucleosides, etc.

In the literature of the last 20 years, innumerable studies on DNA adducts and their determination have been published. These endeavours of a biochemical effect monitoring using DNA adducts have been reviewed several times (Shuker and Farmer, 1992; Hemminki, 1995; La and Swenberg, 1996; Sorsa and Anderson, 1996; Poirier et al., 2000; Farmer et al., 2005; Phillips, 2005) Some reviews comment on the possibilities and limitations of DNA adduct monitoring on hand of special groups of substances like PAH (Angerer et al., 1997; Godschalk et al., 2003), aromatic amines (Sabbioni and Jones, 2002) and dietary heterocyclic amines (Schut and Snyderwine, 1999). Many of these papers described the development of analytical

procedures and their application in in vitro or animal studies. There are only quite a few papers where DNA adducts have been determined in humans (Table 3). In these cases the adducts of alkylating agents, PAH, aflatoxin, products of lipid peroxidation have been quantified.

Out of the huge number of papers on DNA adducts in Table 3(a) only those are listed where DNA adducts have been determined in easily accessible body fluids like blood and urine. There are some groups of substances, which attracted most research activities. These are alkylating substances which occur environmentally and are produced endogenously where alkylguanine and alkyladenine adducts have been determined in blood and urine. The adducts of PAH, frequently called bulky adducts have been used as effect markers of this group of substances which are taken up at workplaces, in the environment and as constituents of tobacco smoke. Ethenoadducts of adenine and cytidine mainly served as indicators of lipid peroxidation products (LPO), though other chemical substances like vinylchloride also yield these products. There are just a few publications which focus on DNA adducts of substances like aflatoxin, dimethylformamide, NO_2, and aromatic amines. Most of the studies shown in Table 3 only investigated some persons or small groups of persons. In many cases it was a purpose of such studies to demonstrate the suitability of DNA adduct measurement for HBM purposes rather than to objective exposure and risk. There are only a few epidemiologic studies where DNA adduct monitoring were included and where dose effect correlations could be gained as in the case of aflatoxin exposure and liver cancer (Qian et al., 1994) or tobacco smoking and lung cancer (Tang et al., 1995).

Table 3

Adduct	Source	Method	Matrix	Reference
(a) DNA adducts in human blood or urine				
BPDE (DNA)	PAH	HPLC-FD; GC-MS	Lymphocytes	Weston et al., 1989
PBDE	Tobacco smoke	^{32}P-postlabelling	BAL	van Schooten et al., 1990
7-Alkylguanines	Dacarbazine; procarbazine; (cancer patients)	^{32}P-postlabelling	WBC	Mustonen et al., 1991
AFB-*N*7-Gua	Aflatoxine	RAI-HPLC	Urine	Groopman et al., 1993
*N*7, *N*2-Alkylguanines	Alkylating agents, environment	GC-MS; GC-MS/MS	Urine	Farmer et al., 1993
3-Alkyladenines	Alkylating agents	Immunoaffinity-GC/MS	Urine	Shuker et al., 1993
3-Alkyladenine	Tobacco smoke; alkylating agents	Immunoaffinity-chromatography-GC-MS	Urine	Prevost and Shuker, 1996
DNA-adduct (bulky)	Aromatic amines	^{32}P-postlabelling	WBC	Zhou et al., 1997
AFB-*N*7-Guanine	Aflatoxin in diet	HPLC	Urine	Yu et al., 1997
AFB(1)-*N*7-Guanine	Aflatoxine in diet	^{32}P-postlabelling	Urine	Poirier, 1997
O-6-Methylguanine	Endogeneous methylating agents	HPLC-ECD	WBC	Kyrtopoulos, 1998
Bulky adducts	PAH, Kuwait-veterans	^{32}P-postlabelling	WBC	Poirier et al., 1998
Bulky adducts	Aromatic amines; tobacco smoker	^{32}P-postlabelling	WBC	Peluso et al., 1998
Bulky adducts	PAH; coke oven; tobacco smoke	^{32}P-postlabelling	WBC; lymphocytes	Binkova et al., 1998
Bulky adducts	PAH; traffic; coal-, wood- burning	^{32}P-postlabelling	WBC	Schoket, 1999
*N*7-HEG	Ethyleneoxide; endogenous	GC/ECD/NCI-HRMS	Lymphocytes	Wu et al., 1999
BP-6-*N*7Gua	PAH; coal smoke	CE-FLNS	Urine	Roberts et al., 2000
Bulky adducts	Tobacco smoke	^{32}P-postlabelling	Sputum	Nia et al., 2000
BP-6-*N*7Gua; BP-6-*N*7Ade	Tobacco smoke; PAH	LC-MS/MS	Urine	Casale et al., 2001
BPDE	PAH	^{32}P-postlabelling	WBC	Schoket et al., 2001
BPDE	PAH	Chemoluminescence; immunoassay	WBC	Divi et al., 2002
5-OH-PhIP	Heterocyclic amine in diet	LC-MS/MS	Urine	Frandsen et al., 2002

Table 3. (*continued*)

Adduct	Source	Method	Matrix	Reference
εAde	LPO	LC-MS/MS	Urine	Gonzalez-Reche et al., 2002
εAde	LPO	GC/NCI-MS	Urine	Chen and Chiu, 2003
PBDE	PAH; occupational exposure	^{32}P-postlabelling	WBC	Lee et al., 2003
εCyd	LPO; ROS	GC-MS	Urine	Chen et al., 2004
Bulky adducts	Diet (PAH?); background exposure	^{32}P-postlabelling	WBC	Palli et al., 2004
BD-6-*N*7Gua	PAH	LC-MS/MS	Urine	Chen et al., 2005
BPDE	PAH; occupational exposure	HPLC/FD	WBC	Mensing et al., 2005
1,*N*-2-ε Gua	LPO; tobacco smoke	LC-MS/MS	Urine	Chen et al., 2005
N-4-Methylcarbamoyl-cytosine	Dimethylformamide	LC-MS/MS	Urine	Hennebrüder and Angerer, 2005
M1dG	ROS	LC-MS/MS	Urine	Otteneder et al., 2006
Nitroguanine	NO_2	Immunoaffinity; chromatography; HPLC/ECD	Urine	Sawa et al., 2006
(b) 8-OHdG levels as biomarker of oxidative stress				
8-OH-dG	Oxidative stress; tobacco smoke	LC-MS/MS	Urine	Renner et al., 2000
8-OH-dG	Oxidative stress	GC-MS	Urine	Mei et al., 2001
8-OH-dG	Oxidative stress	GC-MS	Urine	Liu et al., 2004
8-OH-dG	PAH; oxidative stress	HPLC	Urine	Nilsson et al., 2004
8-OH-dG	Oxidative stress	LC-MS/MS	Urine	Sabatini et al., 2005
8-OH-dG	Tobacco smoking	CE-AD	Urine	Yao et al., 2004

*N*7-HEG: *N*7-(2-hydroxyethyl)guanine; *N*7-MeGua: *N*7-methyl-guanine; BP-6-*N*7Gua: 7-(benzo[*a*]pyren-6-yl)guanine; BP-6-*N*7Ade: 7-(benzo[*a*]-pyren-6-yl)adenine; BPDE: 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydroxybenzo[*a*]pyrene; BP-6-N7Gua: 7-(benzo[*a*]pyrene-6-yl)guanine; 3 εtAde; εAde: 1,N^6-ethenoadenine; 1,*N*2-εGua: 1,*N*-2-ethenoguanine; εCyd: 3,*N*-4-ethenocytosin; 8-oxod Gua: 8-oxo-7,8-dihydroxy-2′-deoxyguanosine; 8-OH-dG: 8-hydroxy-2deoxy-guanosine; AFB-N7-Gua: aflatoxin N7-guanine; RAI: Radioimmunoassay; CE FLNS: capillary electrophoresis fluoresdcence line narrowing spectroscopy; CE-AD: capillary electrophoresis amperometrie detection; LPO: lipid peroxidation; ROS: reactive oxygen species; BAL: bronchoalveolar lavage; WBC: white blood cell; εCyd: 3,*N*-4-etheno-2′-deoxycytine; M1dG: 3-(2-choxy-*β*-D-erythro-pentofuranosyl)pyrinidol[1,2-*α*]purin-IO(3H)one; 5-OH-PhIP: 2-amino-1-methyl-6-(5-hydroxy-) phenylimidazo[4,5-b]pyridine.

Poirier already in 2000 summarized the future of DNA adduct monitoring. She claimed that immunological methods using specific antisera will henceforward provide useful approaches on biomonitoring. Whereas ^{32}P-postlabelling due to shortcomings in specificity will only give an impression of total adduct burden. Fluorescence detection on the other hand is limited to fluorescent adducts like PAH or aflatoxine. All these techniques surely had been and will be helpful for answering special questions. Unless there are strictly specific methods for the determination of DNA adducts yielding results which are comparable and reproducible between the laboratories a broad application of DNA adduct monitoring will be inhibited. Moreover, such methods should enable to prove the chemical structure of the DNA adduct thus adding to elucidate the mechanism of carcinogenesis. To make DNA adducts broadly applicable and a powerful tool of HBM within molecular epidemiology in population studies further endeavours must be made. Analytical methods must be elaborated and optimized which are still more sensitive and specific as those used up till now. Using such methods the spectrum of substances and their DNA adducts must be broadened. These methods must be kept under conditions of quality assurance and must lead to reproducible and comparable results.

8-Hydroxy-2′-deoxyguanosin (8-OHdG)

Besides substance specific DNA adducts, biomarkers of DNA oxidation are increasingly used in HBM (Marczynski and Wilhelm, 2001; Marczynski et al., 2002a, b). Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to DNA at a rate that is probably a significant contributor to the age-related development of cancer. The reactive oxygen species (ROS) can be

physiologically compensated for a large extent by corresponding oxidation–reduction processes. A certain amount of endogenously caused oxidative DNA damage is, however, always present. Additionally, oxidative DNA damage occurs due to exogenous causes, such as ionizing radiation and UV radiation, also many inorganic and organic pollutants or their metabolites induce oxidation—reduction processes, which then, as result of an imbalance between oxidants and antioxidants, lead to oxidative stress. In the presence of oxidative stress, ROS generated in vivo can cause damage to lipids and proteins. DNA, however, is probably the biologically most significant target of oxidative attack. Among all DNA bases, guanine is most prone to oxidation. Upon oxidation a hydroxyl group is added to the 8th position of the guanine molecule and the modified product 8-hydroxy-2′-deoxyguanosine (8-oxodGuo or 8-OHdG) is one of the predominant forms of free radical induced lesions of DNA. The presence of 8-OHdG reveals a lower fidelity in the replication process and enhances the probability of adenine incorporation into the complementary strand, giving rise to G–T transversions. Agents that increase levels of 8-OHdG should thus increase the risk of cancer development. Therefore, 8-OHdG seems a promising tool as an indirect biomarker in HBM of exposure to mutagenic and carcinogenic substances. Nuclear and mitochondrial DNA from tissue and blood lymphocyte is usually the site of oxidation. One suitable biological material for HBM to assess DNA oxidation is WBCs. As new WBCs are continuously being formed in the blood, the adduct levels determined represent only a steady-state level. Taking into account the different lifetimes of the adducts, it must be assumed that short- and long-term effects are included. Measurements of 8-OHdG are also performed in urine. Oxidized DNA repair products are excreted with urine, therefore urinary 8-OHdG is considered to be an important repair product, reflecting the balance between damage and repair rate. Furthermore, because collection is easy and non-invasive, urinary 8-OHdG is regarded also as a suitable biomarker of oxidative stress. However, for interpretation of 8-OHdG levels in urine it should be considered that some 8-OHdG in urine may arise from DNA precursor pool and not from DNA and for other reasons may not reflect oxidative damage to guanosine residues in DNA. With respect to interpretation of 8-OHdG levels in blood cells or urine, it has to be considered that the biological process for the development of cancer is highly complex even after documentation of exposure to mutagenic and carcinogenic chemicals, it is not justified to predict cancer risk based on 8-OHdG measurements. This is underlined by prospective studies which indicate that although DNA adduct is a good biomarker for DNA exposure it is a weak predictor for cancer risk (Au, 2007). It should be noted that Loft et al. (2006) very recently showed an association between 8-OHdG excretion in urine and lung cancer risk among never-smokers, but not among current or former smokers. For 8-OHdG determination sensitive and reliable methods are available. The main method of 8-OHdG determination is HPLC with electrochemical detection (Angerer and Schaller, 1985–2006; Marczynski et al., 2002a, b) and ELISA (Wu et al., 2004). There are many studies now available and a recent review reveals that up to now these data indicate that there is still a lack of well established dose–response relations between occupational or environmental exposures and the induction of 8-OHdG (Pilger and Rüdiger, 2006). Smoking has been identified as a confounder for 8-OHdG, but various occupational studies did not reveal higher levels of 8-OHdG in smokers. Main critical aspects in interpretation of 8-OHdG measurements are related to the analytical challenge, artifactual production of 8-OHdG, inter- and intra-individual variation, confounding factors and inter-laboratory differences. This is underlined by our own studies.

We have investigated the 8-OHdG in PAH exposed workers (Marczynski et al., 2002a, b; 2005) and in mothers living close to a coke oven plant (Hölzer et al., 2005; Wilhelm et al., 2007). The German PAH study revealed that the levels of 8-OHdG in WBCs were significantly increased in workers in graphite electrode and refractory plants when compared to the reference group, but not in workers from other PAH contaminated occupational settings. There was a strong relationship between external and internal exposure to PAH components but the association of internal and external exposure to 8-OHdG was weak or even lacking. In the Hot Spot study North Rhine Westphalia we found increased levels of PAH metabolites in urine and increased 8-OHdG levels in WBCs of mothers living in the vicinity of industrial sources compared to those from a rural area (Hölzer et al., 2005; Wilhelm et al., 2007). However, there were only weak indications of an association between exposure and 8-OHdG in WBCs.

8-OHdG levels as a biomarker of oxidative stress, which may be caused by chemical substances, physical stress, diabetes, or tobacco smoking was applied in many studies. In Table 3(b), only a few of the newest papers are compiled. Diagnostic reliability of this marker is still in debate (Marczynski and Wilhelm 2001). In any case with respect to chemical exposure it is a marker which is unspecific for the substance taken up. That means, if this marker is applied in HBM studies the exposure to a special chemical substance must be objectified additionally. On the other hand, 8-OHdG today can be determined analytically reliably in blood and urine using modern methods of instrumental analysis.

Biomarker of genotoxic effect—comet assay

Among the biomarkers for early biological effects of exposure to environmental mutagenic agents, chromosome aberrations and micronuclei may be the most relevant (Au, 2007). However, in HBM studies the measurement of DNA strand breaks has become very popular (Faust et al., 2004). A huge data basis is now available and Moller (2006a) very recently even suggested the assessment of reference values for DNA damage detected by the comet assay in human blood cell DNA. The comet assay (single-cell gel electrophoresis assay) is a simple and sensitive method for studying DNA damage and repair. Principles, applications and limitations have been summarized by Collins (2004). For HBM usually lymphocytes are prepared from heparinized venous blood samples. Nasal, buccal epithelial cells or urothelial cells centrifuged from urine have also been investigated. A cell suspension is then embedded in agarose on a microscope slide and lysed to liberate the DNA. During electrophoresis under alkaline condition, the DNA fragments migrate to the anode side. The slides are stained with a fluorescent DNA-binding dye. Cells with increased DNA damage display increased migration of chromosomal DNA from the nucleus toward the anode, which resembles the shape of a comet. Data are expressed as tail length (%DNA in the tail) and tail moment (tail length $\times$ %DNA in the tail). The test detects single-strand breaks, alkali labile sites and DNA cross-linking in individual cells. The sensitivity of the alkaline comet assay is greatly influenced by many factors such as pH of the lysis and electrophoresis buffer as well as duration and temperature of electrophoresis. Each lab working with the comet has probably developed its own methodology of the assay, including sampling and storage procedures (e.g. freezing of cells or analysis within a couple of hours after collection). However, there have been many activities to harmonize the comet assay. The present state of validation in HBM (Moller, 2006b) and a suggestion for a standard protocol of the alkaline comet assay (Speit and Hartmann, 2006) has been published recently. Unfortunately, no quality control programs of the assay in multi-laboratory validation trials are available at present.

One main critical issue in using the comet assay in HBM studies is the interpretation of data. It has to be kept in mind that WBCs are surrogate cells, thus the damage detected does not reflect the damage in the target tissue. Additionally, there is a wide intra-individual and inter-individual variability of the comet data since the basal level of DNA damage is influenced by a variety of factors such as lifestyle, diet, infections, medication, air pollution, season, climate or exercise. Thus the predictive value of the comet assay is unknown and it is unjustifiable to assess individual risk of diseases, especially risk of cancer from comet assay results.

Consequently, the use of the comet assay in HBM of individuals that are environmentally or occupationally exposed to genotoxic agents is only recommended for studies on large population samples with different exposure patterns and under consideration of the role played by the above mentioned factors.

A summary of HBM studies using the alkaline comet assay with lymphocytes performed between 2000 and 2003 is given by Faust et al. (2004). Mostly if the corresponding tests were applied, there was a concordance between the comet assay results and those obtained in classical cytogenetic tests such as chromosome aberrations, micronuclei and sister chromatid exchanges. This is confirmed in a recent study performed with 170 healthy volunteers, randomly selected from the general population of the Republic of Croatia (Kopjar et al., 2006). However, Faust et al. (2004) identified some shortcomings in the design of the studies which may partly explain some inconsistency of the results. Especially, although smoking is considered to be a relevant exposure towards various genotoxins, conflicting results have been reported in the comet assay studies (Faust et al., 2004). Many different hypotheses are discussed, but it seems that the reasons for these discrepancies are not known. However, a recent meta-analysis reveals some new aspects. Hoffmann et al. (2005) evaluated 38 studies and found higher levels of DNA damage in smokers than in non-smokers. By subdividing these studies into studies investigating the effect of smoking as a genotoxic exposure (type A studies, $n = 12$) and studies investigating smoking as a potential confounder in occupational studies (type B, $n = 26$) the authors found a significant difference only in type A studies but not in type B studies. Furthermore, studies using image analysis or image length measurements ($n = 23$) only indicated a tendency for a genotoxic effect of smoking, whereas studies using an arbitrary score ($n = 15$) found a significantly higher level of DNA damage in smokers. From his pooled analysis including 125 HBM studies Moller (2006a) showed that when genotoxicity was expressed as % tail DNA the median value was 8.6. Sex and smoking did not influence genotoxicity detected by the comet assay, however, there was a positive association between age and level of DNA damage. On the other hand, duration of alkaline treatment and electrophoresis had no influence.

We have investigated the comet assay in PAH exposed workers and in mother—child pairs living close to a coke oven plant (Hölzer et al., 2005; Wilhelm et al., 2007). The German PAH study (Marczynski et al., 2002a, b, 2005) was introduced in 1999. This cross-sectional HBM study comprised of PAH exposed workers in occupational settings such as a coke-oven plant, a graphite-electrode-producing plant and a manufacture of refractory infeed of converters. The levels of DNA strand breaks were increased in workers

from the manufacture of graphite electrodes and the coke oven plant. There was a strong relationship between external and internal exposure to PAH components but the association of internal and external exposure to the comet assay results was weak or even lacking. In the Hot Spot study NRW we found increased levels of PAH metabolites in urine and increased DNA strand breaks in mothers and their children aged about 6 years living in the vicinity of industrial sources compared to those from a rural area (Hölzer et al., 2005; Wilhelm et al., 2007). However, as in the study with PAH exposed workers, there were only weak indications of an association between exposure and comet assay results, but there was a strong association between DNA strand breaks in mothers and their children (Wilhelm et al., 2007). These results may underline that DNA exposure as measured by the comet assay is not specific for PAH compounds and that synergism with other potential genotoxic chemicals or other DNA stressing factors among the PAH exposed participants probably play an import role.

Analytical methods and quality assurance

In environmental medicine HBM plays an important role to access chronic exposure to chemical agents. Because results of HBM are of great concern to the health of the persons investigated and may cause measures with huge economical consequences the analytical reliability of such results must be guaranteed.

An analytical method consists of four steps:

- Pre-analytical phase,
- Analytical phase,
- Quality assurance,
  - o Internal quality control,
  - o External quality control,
- Evaluation and interpretation of results.

Any of these four steps plays an important role for the reliability of results.

SOPs are an excellent tool to achieve reliable HBM results. This applies especially for the pre-analytical phase were in vivo influences and in vitro interference factors such as time of specimen collection, changes of analyte concentration by degradation or evaporation; exogenous contamination, etc. have an impact on the results. To minimize these effects SOPs are very helpful because standardized procedures of the pre-analytical phase and can be described there.

SOPs on the other side can exactly describe all steps of the analytical procedure such as aliquotation of biological specimen, clean up, calibration, instrumental analysis, etc., so that imprecision and inaccuracy of analytical results can be minimized. The working group Analysis of Hazardous Substances in Biological Material of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the Deutsche Forschungsgemeinschaft (DFG) publishes a collection of SOPs which are tested with respect to the analytical reliability and the reproducibility of the methods. Table 3 shows an overview of the analytical procedures contained in this collection of methods listed according parameters and analytical technique. About 140 methods are available for determining about 200 parameters (Angerer and Schaller, 1985–2006) (see Table 4).

In the analytical phase quality is assessed by internal and external quality control (WHO, 1981). Internal quality control is the systematical monitoring of precision under repeated conditions to determine random errors and the accuracy of quantitative laboratory investigations. In practice it is carried out using commercially available or laboratory made control materials. Such control materials like blood or urine contain defined and constant analyte content. There is a broad spectrum of commercially available control samples from various suppliers like Recipe, München; Nicomed, Oslo; BioRad Laboratories, München which contain most of the biomonitoring parameters shown in Table 1.

External quality assessment is a system for objectively checking the laboratory performance of institutes (WHO, 1981). In the last 30 years, in many member states of the European Union as well as in the USA and in Canada very efficient external quality assessment schemes (EQUAS) have been installed (Table 5) with up to 160 laboratories regularly taking part. These schemes cover all metals which are environmentally relevant (Table 5(a)). Schemes for the determination of organic substances and their metabolites in biological materials are offered in Germany, Canada and Finland (Table 5(b)). With the German scheme proficiency testing of most of the organic pollutants (Table 5(b)) is possible. It has to be pointed out that in each of the schemes of Canada and Germany up to 170 laboratories all over the world are participating between two and six times a year. The rate of successful participation depends amongst others on the parameters and concentrations to be analysed and lay between 52% and 90%. The average success rate is about 70% (Schaller et al., 2002).

The state of the analytical art of HBM in environmental medicine is shown in Table 6. These are the results of the reference laboratories of the German external quality assessment scheme between 1996 and 2006 ($n = 20$). Every biomarker is ideally determined in six reference laboratories on five different days in duplicate. That means, that the imprecision given in Table 6 include the between day and the interlaboratory variation of results. The variation moreover is dependent on the analyte concentration in urine or blood, which in the environmental concentration range

Table 4. Biomarkers and methods in the series "Analyses of Hazardous Substances in Biological Materials/Biomonitoring Methods" of the Deutsche Forschungsgemeinschaft

	Inorganic substances	Organic substances (individual components and substance classes)	Parameters of biological effect
Biomarkers	Metals: Al, As, Ba, Be, Cd, Cr, Co Cu, Hg, In, Mn, Ni Pb, Pt, Sb, Se, Sn, Sr Ti, Tl, V, Zn Anions: CN^-, F^-, Br^-	Haemoglobin adducts: Alkylating compounds Aromatic amines Aromatic nitro compounds VOC metabolites: *t*-,*t*-Muconic acid *S*-phenylmercapturic acid Aromatic carbonic acids Alkoxycarbonic acids Organochlorine compounds: HCH, HCB, DDE, aldrin, etc. PCDD, PCB PAH metabolites Pesticide metabolites: Organophosphates Pyrethroids Carbamates Phenoxycarbonic acids Phenols, catechols Phthalates: DBP, BBzP, DEHP, DiNP, etc. Perfluorinated compounds: PFOA, PFOS	Acetylcholinesterase β2-Microglobulin Cholinesterase CO-Hb δ-Aminolevulinic acid δ-Aminolevulinic acid *dehydratase* Erythrocyte porphyrins Methaemoglobin
Analytical methods	F-AAS; GF-AAS; hydride-AAS; cold vapour AAS; inverse voltammetry; ICP-OES; ICP-MS; ion-selective electrodes	GC-FID; GC-ECD; GC-TID; GC-MS; HPLC-MS/MS; head-space gas chromatography; HPLC; photometry; fluorimetry	Fluorimetry; photometry; gas chromatography; enzyme-linked immunoassay; radioimmunoassay

goes down to 100 ng/l. Table 6 shows that even such low concentration can be measured by highly skilled laboratories with a between laboratory imprecision of less than 19%. About 60% of the biomarkers can be determined within an imprecision below 15%. Another message of Table 6 is that HBM in environmental medicine is under control. HBM data are reliable.

Thus, nowadays using SOP, internal and external quality assurance can guarantee that HBM results have a very high level of reliability. HBM can be used for the assessment of internal exposure and can be used as a basis for further decisions. It has to be pointed out that ambient monitoring for instance of air, soil, house dust, etc. cannot refer on such measures of quality assurance. This refers to SOP and especially to external quality assessment. In other words, there are hardly any data about the reliability of ambient monitoring data.

Exponentially increasing numbers of publications show HBM to be an expending discipline (Needham et al., 2007). New pollutants enter the scene like perfluorinated compounds (PFCs) or secondary phthalate metabolites, etc. In such cases emerging analytical gaps are rapidly closed by the leading laboratories in this field. The same is true for external quality assessment. This development is based on the availability of highly sophisticated methods of instrumental analysis like atomic absorption spectroscopy (AAS), inductively coupled plasma–mass- spectrometry (ICP-MS), gas chromatography-mass spectrometry (GC-MS), gas chromatography-tandem mass spectrometry (GC-MS/MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), etc. This ever-growing analytical expenditure affords huge financial investments. This will lead to a concentration process of HBM laboratories with only some centres of excellence that are able to ride on the crest of the wave. In spite of this it will pay to reach the ultimate summit of HBM, because it is the only way to identify and to quantify human exposure and risk, elucidate the mechanism of toxic effects and to ultimately decide if measures have to be taken to reduce exposure. Risk assessment and risk management without HBM lead to wrong risk estimates and cause inadequate measures.

Table 5. External quality assessment schemes (worldwide)

Country	Blood	Urine	Serum/Plasma	Reference
(a) Metals				
Belgium	As, Cd, Hg, Mn, Pb		Al, Cu, Se, Zn	www.iph.fgov.be
Canada	Cd, Hg, Pb	As, Cd, Cr, Cu, fluoride, Hg, Pb, Se, Zn	Al, Cu, Mn, Se, Zn	www.ctq.qc.ca
France			Cu, Se, Zn	www.inrs.fr
Germany	Cd, Co, Cr, Hg, Mn, Ni, Pb	Al, AlA, As (total, inorganic, species), Be, Cd, Co, Cr, creatinine, Cu, fluoride, Hg, Ni, Sb, Tl, V, Zn	Al, Cr, Co, Cu, Mn, Ni, Pt, Se, Zn	www.G-EQUAS.de
Great Britain	As, Cd, Hg, Mg, Mn, Pb, Zn	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Tl, Zn	Al, Cu, Se, Zn	www.surrey.ac.uk/sbms/egas
Italy	Cd, Pb	As, Co, Cr, Mn, Ni, Pb, Tl	Al, Cu, Se, Zn	www.iss.it
Netherlands	Cd, Co, Hg, Pb, Se, Tl	Al, Cd, Co, Cu, Hg, Mg, Pb, Se, Tl, Zn	Al, Co, Cr, Cu, Li, Mg, Mn, Se, Zn	www.skzl-mca.nl
Spain	Pb	Hg, Cr		www.mtas.es/insht
USA	As, Cd, Hg, Pb, Protoporphyrin (ery)	As, Ba, Be, Cd, Co, Cs, Hg, Mo, Pb, Pt, Sb, Tl, U, W	Al, Cu, Se, Zn	www.wadsworth.org/testing/lead/ptprogram.htm
(b) Organic substances				
Canada			PCB: 28, 118, 138, 153, 170, 180 β-HCH *p,p′*-DDE, *p,p′*-DDT HCB, trans-nonachlor, Oxychlordane, *PBDE # 47, 99,* parlar # 26, 50 total lipids	www.ctq.qc.ca
Finland		Creatinine, 2,5-hexanedione mandelic acid, methylene dianiline, methylhippuric acid, *t,t*-muconic acid, 1-/2-naphtol, phenol, thiocyanate, trichloroacetic acid		www.ttl.fi/fioh
Germany	Benzene Dichloromethane 1,2-Dichloroethane Ethylbenzene Tetrachloroethene Toluene Trichloroethene Xylenes	Acetone Methanol Methylethylketone Butoxyacetic acid Ethoxyacetic acid 2,5-Hexandione Hippuric acid 5-Hydroxy-*N*-methylpyrrolidone 2-Hydroxy-*N*-methylsuccinimide 1-Hydroxypyrene Mandelic acid *N*-methylformamide Methylhippuric acids *t,t*-Muconic acid Phenol Phenylglyoxylic acid *S*-phenylmercapturic acid 2-Thio-thiazolidine-4-carboxylic acid Trichloroacetic acid Pyrethroide metabolites: Br_2-CA, *cis*-Cl_2CA, *trans*-Cl_2CA, 3-PBA Alkyl phosphates 2,5-Dichlorophenol 2,4,6-Trichlorophenol Nicotine Cotinine Pentachlorophenol Phthalate metabolites	*p,p*-DDT *p,p*-DDE HCB, α, β, γ-HCH PCB: 28, 52, 101, 138, 153, 180 PFOS, PFOA Pentachlorophenol	www.G-EQUAS

Table 6. Imprecisions for the determination of biomarkers by the reference laboratories of the German external quality assessment scheme (G-EQUAS 1996–2006)

Matrix	Analytes	Unit	Concentration range of assigned values	Precision of reference laboratories[a] C.V. (%)
Inorganic substances				
Blood	Pb	μg/l	25–160	11–5
	Cd	μg/l	0.4–3.0	16–8
	Hg	μg/l	1.1–6.8	16–6
Urine	As	μg/l	8–39	13–7
	Cd	μg/l	0.4–4.7	19–8
	Cr	μg/l	0.6–4.5	17–8
	Ni	μg/l	1.4–10.5	14–6
	Hg	μg/l	1–5.3	16–7
Organic substances				
Urine	1-HP	μg/l	0.3–1.5	18–8
	Br_2-CA	μg/l	0.3–6.6	16–7
	cis-Cl_2-CA	μg/l	0.5–8.9	16–8
	trans-Cl_2-CA	μg/l	0.6–9.8	17–7
	3-PBA	μg/l	1.1–16.0	14–7
	2,5-Dichlorophenol	μg/l	9–59	12–1
	2,4,6-Trichlorophenol	μg/l	2–91	15–8
	PCP	μg/l	1.5–16.5	15–7
	Dimethylphosphate	μg/l	18–190	19–6
	Dimethylthiophosphate	μg/l	15–550	13–7
	Dimethyldithiophosphate	μg/l	7–91	8–22
	Diethylphoshate	μg/l	8–91	12–9
	Diethylthiophosphate	μg/l	10–59	16–10
	Diethyldithiophosphate	μg/l	7–56	18–10
	Cotinine	μg/l	30–1250	9–5
	Nicotine	μg/l	30–1220	9–7
	5-OH-MEHP	μg/l	10–230	10–7
	5-Oxo-MEHP	μg/l	8–220	10–6
	5-Carboxy-MEPP	μg/l	15–330	10–5
Serum	p,p'-DDE	μg/l	0.3–8.2	15–9
	HCB	μg/l	0.2–7.5	15–6
	α-HCH	μg/l	0.2–2.7	14–10
	β-HCH	μg/l	0.15–3.2	17–9
	γ-HCH	μg/l	0.15–2.6	17–8
	PCB-28	μg/l	0.2–3.3	19–10
	PCB-52	μg/l	0.2–3.0	16–10
	PCB-101	μg/l	0.2–5	17–6
	PCB-138	μg/l	0.2–5.7	17–8
	PCB-153	μg/l	0.2–10	15–8
	PCB-180	μg/l	0.2–3.3	16–8
	PCP	μg/l	1.9–13.8	13–6
	PFOA	μg/l	15–51	8–5
	PFOS	μg/l	28–52	7–6

Environmental concentration range.

[a]Six reference laboratories; each laboratory five duplicate determinations on five different days.

Interpretation of HBM data: reference and limit values

For the evaluation of HBM data clear criteria are necessary. In Germany, the HBM commission of the Federal Environmental Agency recommends two different kinds of criteria (a) reference values and (b) human biomonitoring values (HBM values) (Ewers et al., 1999; Schulz et al., 2007b). The reference values are intended to characterize the upper margin of the current background exposure of the general population to a given environmental pollutant. It is important to emphasize that reference values do not represent toxicologically derived biological exposure limits. Reference values are just a statistical description of the background exposure of a certain population, in this case of the German population. Up to now the German HBM commission has evaluated reference values for six of the most important environmental metals (arsenic, lead, cadmium, mercury, nickel and platinum). Moreover,

reference values have been evaluated for several groups of organic substances like polychlorinated biphenyls, organochlorine compounds, polyaromatic hydrocarbons, phthalates, organophosphates and pyrethroids. More details are reported by Schulz et al. (2007b) in this issue. Biomonitoring population studies have also been conducted in other countries which could be used to evaluate such reference values. This especially applies to the NHANES studies in the USA where some thousands of persons are investigated with respect to their internal exposure to 148 chemical substances (CDC, 2005).

The German HBM values on the contrary represent health related biological exposure limits, which are derived from toxicological and epidemiological studies. HBM I is the concentration of an environmental toxin in a human biological material below which there is no risk of adverse health effects in individuals of the general population. HBM II is the concentration of an environmental toxin in a human biological material above which there is an increased risk of adverse health effects. That up to now only for lead, cadmium, mercury and pentachlorophenol health-based limit values (HBM values) could be established shows the difficulties to deduce such values. More details are reported by Schulz et al. (2007b) in this issue.

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