Biomarkers of exposure to carcinogens

Paul T.J. Scheepers

Research Lab Molecular Epidemiology, Department for Health Evidence, Radboud university medical center, Nijmegen, The Netherlands

In human exposure assessment a biomarker is a parent substance, metabolite or addition product (adduct) that can be measured in blood, urine, exhaled air, or in other biological tissues. The biomarker provides information on the internal exposure to a chemical substance. Biomarkers can be used to evaluate exposure to carcinogenic substances on the workplace or in the general environment. If the parent compound is used as a biomarker, information on the uptake different sources and via different routes of uptake can be obtained. Use of a metabolite or adduct to DNA or proteins also provides information on bioavailability and metabolic activation. The half life of biomarkers determines the moment of sample collection relative to time of exposure. Some biomarkers are mechanism-based and provide useful information on the formation of genotoxic intermediates. Most biomarkers reflect systemic bioavailability rather than a target dose, which sometimes limits the interpretation in terms of assessment of cancer risk. The method of sample collection, pre-treatment, transportation, storage and a reproducible and sensitive method of analysis should be selected with care. Background values and biological limit values provide a framework for interpretation. The study protocol should comply with international standards in ethics of biomedical studies involving human subjects.

Key words: Human biological monitoring, blood, urine, end-exhaled air, adduct, urinary metabolite, biological limit value
73.1. **Introduction**

In human exposure assessment a biomarker is a parent substance, metabolite or addition product (adduct) that can be measured in blood, urine or exhaled air. Sometimes hair, skin and nails are also used as tissues for isolation of biomarkers. For isolation of DNA-adducts there is some experience with the use of exfoliated epithelial cells isolated from the buccal mucous or from urine. A biomarker provides information on the internal exposure to a substance. In contrast to external exposure monitoring that can be provided by personal air sampling or skin contamination testing.

The use of biomarkers in a human biological monitoring campaign can be a good way to evaluate exposure to carcinogenic substances on the workplace or in the general environment.

If the parent compound is used as a biomarker of exposure, information on uptake of a substance from various different sources and via different routes of uptake can be obtained. Use of a metabolite or adduct to DNA or proteins will also provide information on bioavailability and metabolic activation.

Even if the biomarker is not identical to the substance a person is exposed to, it may still contain chemical structure information such as in an addition product that can be linked to this parent substance. This makes a biomarker very useful for assessment of exposure. The half life of biomarkers determines the moment of sample collection relative to time of exposure.

Elimination from the lungs is usually fast (minutes to hours), elimination from plasma and urine is fast (hours to days), elimination from blood cells is much longer (weeks to months) and the half life of adducts to macromolecules depends on many factors but may vary from hours to days for most DNA-adducts to weeks-months for some specific DNA-adducts and their repair products (Henderson et al., 1989). Adducts to proteins may have a longer lifespan (weeks to months), depending on the turn-over of the protein or of the cell such as in the case of haemoglobin in erythrocytes (Scheepers, 2008).

It must be noted that the excretion pattern is not just dependent on the terminal half life of a substance but is also dependent on the pattern of exposure and the porte d’entrée. Biomarker levels will show a different pattern in workers as opposed to subjects exposed in the general environment. Inhalation exposure will show a pattern of excretion that is different from that after dermal absorption.

There are many specific chemicals that are very persistent due to specific properties like a high lipophilicity or non-covalent binding to proteins (e.g. halogenated substances like PCBs, dioxins and also some metals like cadmium and lead).

Metabolites and adducts are usually mechanism-based and provide useful information on the formation of meta-stable intermediates that are involved in genotoxic activity. Most biomarkers reflect systemic bioavailability rather than a target dose, which sometimes limits the
interpretation in terms of assessment of cancer risk. Some biomarkers have been used to study exposure to carcinogenic substances in the general population or reconstruct exposures after chemical incidents involving carcinogenic substances. Based on evaluations by the International Agency for Research on Cancer (IARC) to 2003 it is estimated that workers are exposed to carcinogenic substances in 18 occupations and industries. These exposures involve 28 substances classified as definite human carcinogens, 27 probable carcinogens, and 113 agents classified as possible occupational carcinogens (Siemiatycki et al, 2004).

In this chapter parent compounds will be discussed in section 1, metabolites in section 2, adducts to proteins in section 3, and DNA-adducts and their products of enzymatic repair in section 4. In section 5 the use of biomarkers in human biological monitoring studies will be discussed.

73.2. PARENT COMPOUNDS

For those substances that can exert their toxicity without any metabolic activation a parent compound is suitable as a biomarker. For the large group of volatile organic compounds (VOC), the primary toxic effect may be neurotoxicity, whereas a metabolite may represent a genotoxic substance. For this reason parent substances are often determined as relevant biomarkers of exposure in body fluids and exhaled air. Biological monitoring of metals will also be discussed.

73.2.1. Body fluids

Volatile organic compounds (VOC) can be determined from blood by headspace analysis. In this analysis the sample the analyte is exchanged between the liquid and gas phase until equilibrium. In the gas phase the concentration is determined and the concentration in the liquid is then calculated using the blood-air partitioning coefficient. The advantage of such an approach is a clean matrix for gas chromatographic analysis. Parent organic compounds and inorganic compounds can also be analysed from urine if they are readily soluble in water. An important group of carcinogens that is normally determined as a parent compound from body fluids is the group of metals (Nordberg et al., 2007).

73.2.1.1. Blood

Most organic substances can be analysed from full blood or plasma. For volatile organic substances a head-space analysis may be used. In plasma metals can be free or bound such as to metallothioneins. Metals may also be determined from different types of blood cells such as erythrocytes and granulocytes. An interesting application is the determination of chromium from erythrocytes e.g. in workers who are exposed chromate-containing primers or by inhalation of fumes from stainless steel welding. Chromates
containing hexavalent chromium may enter blood cells using ion channels (Figure 1). In this way chromium becomes trapped in the erythrocyte and the systemic exposure to hexavalent chromium can be evaluated by isolation of the erythrocytes and determination of the total chromium content. Also the plasma levels of chromium in welders are related to the type of metal that is welded and the composition of the electrodes used (Scheepers et al., 2008).

Figure 1: Cr VI enters the erythrocyte through an ion transporter for phosphate and sulphate, in the red blood cell Cr VI is reduced to Cr III by Fe II in oxy-haemoglobin that is converted to met-haemoglobin. Cr III cannot pass the membrane, causing accumulation of chromium in the cell (Lewalter et al., 1985).

73.2.1.2. Urine

Urine can also be analysed for the parent substance such as in the case of organic solvents (Fustinoni et al., 1999) but if the carcinogenicity involves enzymatic activation a downstream metabolite may be selected as a biomarker (see section 2). Urine is also a good matrix for detection of metals. Some knowledge about speciation and kinetics is needed in order to interpret urinary metal excretion.

In the analysis of arsenic speciation between inorganic arsenic and organic is useful because of differences of the origin and of toxicity. Inorganic As can be derived from drinking water and is a known human carcinogen, whereas organic arsenic compounds with a much lower toxicity originate primarily from the diet, in particular seafood (Buchet et al., 1996).

In biological monitoring of chromium kinetics are important. Stainless steel welders are exposed to welding fume particles that contain chromates. Chromium leaches very slowly into the circulation due to the sparse water solubility of some of the chromates that are deposited deeply into the lungs (Schaller et al., 2007). This explains why the pre-shift urine sample of a welder may still contain considerable levels of chromium compared to a non-exposed worker (Scheepers et al., 2008). In this case exposure during the work shift can be evaluated by comparing pre-shift and post-shift values of urinary chromium excretion.
Figure 2: Exposure to benzene can be investigated using breath analysis. End-exhaled air was collected by children attending primary school in Turkey. The volatile organic solvents are transferred to a solid phase by the investigator directly after sample collection. In this way the organic compounds are well preserved for later analysis. (Photo by J. Konings)

End-exhaled air is defined as the last 300-400 mL at the end of a prolonged exhalation and is equivalent to the alveolar fraction. It can be easily and non-invasively collected in both adults and children. In this air volume the chemical substance equilibrates almost instantly with arterial blood, ruled by the blood-air partition coefficient. With this parameter the concentration in the arterial blood flow that takes the substance to the brain can be calculated. End-exhaled air can be collected in a ~ 100 mL container. This container must be equipped with a one-way valve to prevent ambient air being drawn in if the subject starts to inhale at the end of the deep exhalation. There are commercial systems such as the BIOVOC that can be used for the routine collection of end-exhaled air samples in adults and children (Figure 2). In the analysis compared with body fluids, exhaled air is a very clean matrix that enables ultrasensitive detection of VOC down to pmol/L for chlorinated hydrocarbons.
73.3. METABOLITES

Biotransformation leads to detoxification for most chemical substances. However, metabolism may also lead to formation of reactive intermediates and more or less stable metabolites that can exert a specific toxic effect that may be different from the toxicity of the parent compound. As a parent compound styrene can be determined in end-exhaled air or urine (Teixeira et al., 2007). Styrene is metabolised to styren-7,8-oxide, which is a genotoxic metabolite. Styrene-7,8-oxide and phenylethleneglycol can be determined in blood (Christakopoulos et al., 1993). In urine mandelic acid, phenylglyoxylic acid, phenylglycine and 4-vinylphenol are possible urinary biomarkers (Figure 3). It is also possible to determine the secondary metabolites 1- or 2-phenyl-2-hydroxyethylmercapturic acid derived from a glutathione conjugate (Fustinoni et al., 2008). Some metabolites or conjugates are less useful because they are not specific for styrene. E.g. benzoic acid is not specific for styrene exposure since this substance also used as a food additive (E210). Hippuric acid, besides being a reaction product of benzoic acid, is also a conjugate formed following exposure to toluene.

Figure 3. Part of the biotransformatin pathway of styrene. This cheme shows the many options for selection of bioarkers (Fusinoni et al. 2008).
73.4. PROTEIN ADDUCTS

Peptides and proteins contain numerous nucleophilic groups that can be targets for electrophilic attack by reactive intermediates to form adducts (Figure 4). These are mostly electrophilic but not only alkylating substances. Törnqvist et al. (2002) point out that not all of these adducts are exclusively of post-translational origin like N-terminal val that are much used as biomarkers (Table 1).

Intracellular proteins such as hemoglobin (Hb) follow zero-order kinetics, leading to a steady state level that is determined by the turnover of the cells. In the case of Hb the life span of the erythrocytes is 126 days in humans (Bishop, 1964), causing a linear decrease of the adduct after cessation of exposure with a half life of 63 days in humans. This kinetic pattern was observed in studies following the adduct levels after withdrawal from smoking, although the levels of 4-ABP-Hb adducts declined faster than expected (Maclure et al. 1990). The formation and decay of adducts to proteins free in solution such as serum albumin (SA) follows first-order kinetics. For adducts to SA in humans this results in a half life of 20 days (Bishop, 1964). Adducts to SA and Hb follow these kinetics as long as they are chemically stable. So far a mechanism of enzymatic repair of adducts to SA and Hb was not reported. Some studies have shown the formation of adducts to proteins with a longer life span than SA and Hb such as histones. So far these proteins have not been detected in humans in vivo (Scheepers et al., 2008).

Figure 4. Hypothetical peptide showing amino acids in their predominating form at pH ~7. Atoms shown in bold represent the bases with nucleophilic reactivity. In this peptide valine is at the N-terminal position. Modified from Törnqvist et al., 2002.
Table 1: Substances for which haemoglobin adducts were observed in humans (based on Törnqvist et al., 2002).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Adduct</th>
<th>Involved amino acid</th>
<th>Background (pmol/g globin)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non-smokers</td>
<td>Smokers</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Acetaldehyde</td>
<td>Lys</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>N-2-carbamoylethyl</td>
<td>N-term val</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>N-2-cyanoethyl</td>
<td>N-term val</td>
<td>&lt;2</td>
<td>80</td>
</tr>
<tr>
<td>3-Aminobiphenyl</td>
<td>3-nitrosoobiphenyl</td>
<td>Cys</td>
<td>ND</td>
<td>14.1</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>4-nitrosoobiphenyl</td>
<td>Cys</td>
<td>44.3</td>
<td>220</td>
</tr>
<tr>
<td>Benzene</td>
<td>S-(phenyl)</td>
<td>Cys</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,3-Butadiene</td>
<td>N-(2,3,4-trihydroxybutyl)</td>
<td>N-term val</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chrysene</td>
<td>r-1,t-2,3,t,c-4-tetrahydroxy-1,2,3,4-tetrahydrochrysine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N,N-dimethylformamide</td>
<td>N-methylcarbamoyl</td>
<td>N-term val and lys</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NNK(^b)</td>
<td>4-hydroxy-1-(3-pyridil)-1-butanone</td>
<td>Asp, glu or terminal carboxylate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>N-2-hydroxyethyl</td>
<td>N-term val</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Glycidol</td>
<td>2,3-dihydroxypropyl</td>
<td>N-term val</td>
<td>2-7</td>
<td>~10</td>
</tr>
<tr>
<td>Propylene oxide</td>
<td>2-hydroxypropyl</td>
<td>N-term val</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Styrene</td>
<td>S-2-hydroxy-2-phenylethyl and 2-hydroxy-1-phenylethyl</td>
<td>Val</td>
<td>2.59(^c)</td>
<td>2.59(^c)</td>
</tr>
<tr>
<td></td>
<td>2-hydroxy-2-phenylethyl and 2-hydroxy-1-phenylethyl</td>
<td>Cys</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) -, unknown; \(^b\) nitroamine-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; \(^c\) smokers and non-smokers
73.5. DNA ADDUCTS AND DNA-REPAIR PRODUCTS

73.5.1. Addition products

Similar to proteins, the DNA-bases also contain nucleophilic targets for covalent binding of electrophilic intermediates (Figure 5). For a long time sensitive detection of these products relied on isolation of DNA from peripheral lymphocytes. Some attempts have been successful to use other tissues for isolation of DNA-adducts, such as exfoliated bladder epithelial cells that are excreted with urine (Talaska et al., 1996) and exfoliated epithelial cells that can be retrieved from swipes of buccal mucous (Romano et al., 1999). With $^{32}$P-postlabelling it is possible to detect 1 adduct in $10^8$ nucleotides. Identification of the adducts relies on co-elution of the isolated adduct from human blood samples with laboratory-synthesised adduct standards, preferably using a liquid chromatography system for separation. Nowadays the sensitivity of mass spectrometry (MS)-based techniques is in the same range as for the $^{32}$P-postlabelling method (Singh et al., 2006).

Figure 5: General structure the DNA with the four DNA-bases and R representing the xenobiotic adduct forming substance in the case of a phosphotriester adduct.

In contrast to protein adducts most DNA structure modifications are not stable due to the activity enzymatic repair (base and nucleotide excision repair, O$^6$-alkylguanine-DNA alkyltransferase, glycosylases, etc). This means that some adducts may disappear after several hours to a few days. However, some bulky adducts such as adducts formed by polycyclic
aromatic hydrocarbons (PAH) may be more stable. These adducts have been successfully used to characterise both occupational (Van Schooten et al., 1995, Binkova et al., 1997, Hemminki et al., 1997) and environmental exposures (Hemminki et al., 1990; Perera et al., 2005) to PAH. These DNA adduct levels also correlate well with tobacco smoking (Phillips et al., 1988, Talaska et al., 1991).

Phosphotriester-adducts appear also more persistent because there are no known enzymatic repair systems in eukaryotic cells and the development of methods for this type of adducts could yield interesting novel biomarkers for long-term exposure (Scheepers, 2008).

### 73.5.2. Products of DNA-repair

After repair N-7-guanine and N-3-adenine adducts are excreted in urine, which offers an opportunity to use these enzymatic repair products as biomarkers of short-term exposure. Adducts of ethylene oxide to N-7-guanine have been chemically characterised by LC-ESI-MS/MS (Marsden et al., 2007). Such repair products have also been used to characterise exposure to aflatoxin B1 (Ross et al., 1992, Qian et al., 1994). If determined in urine these products do not provide information on the tissue where the adduct was formed.

### 73.5.3. Other approaches

Reactive oxygen species can be formed during inflammatory processes resulting from exposure to fine particles. Some reactive intermediates of chemical exposures may also be involved in redox cycling or auto-oxidation, generating reactive oxygen species. Especially when transition metals are also present, reactive hydroxyl radicals can be formed. Such events can cause oxidative damage to DNA that is normally effectively repaired by nucleoside and base excision repair enzymes but a residue of repair products may be excreted. These repair products can be used as biomarkers for oxidative damage. Of several repair products 8-hydroxydeoxyguanine (8-OHdG) has been most used as a biomarker. This approach is valuable to assess general exposure to oxidants but cannot be used to study the contribution of specific agents (Battershill et al., 2008). Chromosome aberrations, sister chromatid exchanges (SCEs), and micronuclei can be related to cancer but they are not specific to a specific chemical exposure. Also the determination of urinary mutagenic activity in the Salmonella typhimurium assay (Bos and Jongeneelen, 1988) is nonspecific to the causing agents and therefore not suitable in most studies of exposure assessment.
73.6. GUIDELINES FOR HUMAN BIOLOGICAL MONITORING OF EXPOSURE TO CARCINOGENIC SUBSTANCES

73.6.1. Study design

73.6.1.1. Research question

Human biological monitoring can be used for scientific research, routine monitoring or to find answers to *ad hoc* questions. Scientific research can be done to validate a biomarker in a volunteer study, i.e. study of kinetics of excretion of a biomarker or a field validation to assess the suitability of a biomarker for the purpose of exposure characterisation. Once validated, biomarkers can be used as quantitative exposure parameter in epidemiological studies. Well validated biomarkers can be used in long-term monitoring programmes to reveal trends in exposure that can help to support policy makers. Examples of such programmes are NHANES and GerES (see list of references for web-addresses of these projects). Biological monitoring can also be used to reconstruct exposure after chemical incidents in industry or any incidents that are marked by unintended release of chemicals into the environment. Biomarkers of exposure can also be used to evaluate the effectiveness of remedial actions on a workplace such as a change in work procedures or introduction of personal protective equipment.

73.6.1.2. Study population

The study population may be selected at random from the general population. In occupational settings the subjects who are at risk are often clearly identifiable by their job title. For some study aims it may be useful to compare two groups that are different with respect to one or more determinants of exposure or that may be different in health status. Before selecting study subjects, a list of inclusion and exclusion criteria should be drafted.

73.6.2. Design

73.6.2.1. Follow up study

Sequential observations can be a good way to find out about work-related sources of exposure. In the setting of occupational exposure a pre-exposure or background value may be obtained on the morning before the start of the first shift of a workweek (pre-shift spot sample) and subsequent samples may be collected on the same day or on subsequent days during a working week, including a post-shift sample at the end of...
the workweek. Using this design a great number of individuals can be followed parallel in time. This strategy can be extended by including reference subjects. The reference subjects should have characteristics very similar to the subjects from the index group (age, social economic status, gender, smoking status) but with a suspected lower exposure. In practice persons with mild but specific health complaints may be compared with persons that did not report any of these symptoms to study a possible role of chemical exposure.

An intervention study is often used to evaluate a change in time. This can be a change in the determinants of emissions of toxic substances or a change in the determinants of exposure. In an intervention study the initial situation (before the change) is compared to a new situation, using well-standardised methods. To rule out a temporal covariate, it is recommended to use a cross-over design. Two possible designs for intervention studies are illustrated in Figure 6.

Figure 6: Study design to evaluate a transition from situation A to situation B. All workers can be followed at the same time (a). In order to rule out the influence of a temporal covariant (e.g. season or workday in the week) or the effect of sequence (e.g. a learning effect), the population can be randomly divided in subgroups (b). Each worker is assigned to one of the groups at random. Half of the population is followed in the transition from situation A to B, whereas the other half is followed in a transition from B to A.

### 73.6.2.2. Confounders and effect-modifier

Besides the exposure to a carcinogenic substance, several host factors may have an influence on the biomarker level in an individual (Table 2). Some dietary factors may have a direct influence as sources of (co-) exposure. For other factors it is possible to adapt the study design. E.g. the results in smokers and non-smokers can be analysed in separate
groups. However, if factors are less frequent or the influence on biomarkers is not well understood, it is recommended to reduce their influence by standardisation.

Table 2: Factors that may influence the observed level of a biomarker.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Influence</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circadian rhythm</td>
<td>Temporal variation in composition of body fluids</td>
<td>Standardise times of sample collection</td>
</tr>
<tr>
<td>Body fat</td>
<td>Influence on toxicokinetics</td>
<td>Register body mass index or a more specific measure of body fat</td>
</tr>
<tr>
<td>Meal</td>
<td>Influence on blood lipid content</td>
<td>Collect samples before rather than after a meal</td>
</tr>
<tr>
<td>Smoking</td>
<td>Direct influence on levels of several biomarkers (sometimes indirect by induction of metabolic activity)</td>
<td>Study influence of smoking in a separate group and standardise for this effect. Register the amount smoked/day</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Inhibition or induction of metabolic activity</td>
<td>Register the number of alcoholic beverages/day</td>
</tr>
<tr>
<td>Medication</td>
<td>Inhibition or induction of metabolic activity</td>
<td>Register use of prescribed and non-prescribed medication</td>
</tr>
<tr>
<td>Diet</td>
<td>Direct influence on levels of several biomarkers</td>
<td>Register use of certain food components, standardise by asking volunteers to refrain from consumption of certain specific food components</td>
</tr>
<tr>
<td>Food supplements</td>
<td>Direct influence on levels of several biomarkers</td>
<td>Register the use of certain specific food supplements like creatine monohydrate</td>
</tr>
<tr>
<td>Physical exercise</td>
<td>Influence on toxicokinetics</td>
<td>Register level of physical exercise and evaluate impact by PBPK model</td>
</tr>
</tbody>
</table>

73.6.2.3. Questionnaires

The characteristics of study subjects can be registered in a questionnaire. Information about gender, age and biometry of the subjects as well as some questions about physical exercise, diet, and alcoholic beverages, and use of medication is obtained using standardised questions. Sometimes outliers in biomarker levels can be explained using the information from the questionnaire. Table 3 contains examples of some co-exposures that could interfere with the primary exposure of interest. In
the case of collection of repeated spot urine samples, breath samples or blood samples some questionnaires have to be completed (repeatedly) at each moment of sample collection.

Table 3: Possible sources of co-exposure in the general population.

<table>
<thead>
<tr>
<th>Chemical marker</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antineoplastic agents</td>
<td>Treatment with these agents</td>
</tr>
<tr>
<td>(total) Arsenic</td>
<td>Consumption of sea-food</td>
</tr>
<tr>
<td>Chlorinated hydrocarbons</td>
<td>Evaporation from recently dry cleaned clothes</td>
</tr>
<tr>
<td>Nickel/Chromium</td>
<td>Orthopaedic or dental implants</td>
</tr>
<tr>
<td>PAH</td>
<td>Treatment of psoriasis or eczema with coal tar ointment or who use coal tar containing shampoo; consumption of broiled, fried, smoked and barbecued meat and fish</td>
</tr>
<tr>
<td>Phthalates</td>
<td>Leaching from plastics</td>
</tr>
<tr>
<td>Platinum</td>
<td>Exhaust fumes from gasoline-powered vehicles with platinum-containing three way catalysts</td>
</tr>
</tbody>
</table>

73.6.3. Ethics issues

In this section some general guidelines are given regarding ethics issues that need consideration during the process of preparation and performance of human biological monitoring studies. Information will be provided for the study protocol that is needed in order to obtain approval from the ethics committee that is responsible for the evaluation of scientific studies involving human subjects.

73.6.3.1. Informed consent

An information and consent procedure is normally used in biomedical research to inform human subjects about the purpose of the study, their role in the study and their rights (e.g. the possibility to discontinue their participation and their own and external access to personal data). This procedure should be in line with the declaration of Helsinki (World Medical Association, 2000).

73.6.3.2. Sample coding

Coding is a measure to ensure confidential handling of information, protecting the individual and enabling blinded analysis. It should be specified by whom, where and how the samples are coded, how the code forms are kept in an archive and what links between code and study outcome exist (Knudsen, 2004, 2005). Persons who have access to the code list must be identified. It should also be clear for how long the code lists will be kept and who is responsible for their destruction.

73.6.3.3. Protection of privacy of study subjects
During the study the privacy of the study subjects should be respected. Normally, the information identifying a person will be kept in the medical file by the physician directly responsible for the medical health of the study persons (occupational physician or general physician). Also databases containing the results from questionnaires should be made anonymous. If explicitly requested individual study subjects will be granted access to their individual study results.

73.6.3.4. Obtaining ethics approval

To obtain ethics approval a request should be submitted to a national, regional or local review committee recognised by the (health) authorities. The purpose of the study and the study protocol should be outlined. The study protocol includes the method of recruitment of study subjects, inclusion/exclusion criteria of study subjects, description of activities that may lead to exposure to specific carcinogenic substances studied, study design, specification of the analytes, future use of samples and protection of privacy of the study persons. Usually all information and documents that will be provided to the study persons should also be reviewed by the ethics committee.

73.6.4. Communication

73.6.4.1. Introduction of the study

Depending on the purpose of the study and the size of the study population, a study can be introduced personally by a physician or during a meeting by oral presentation. It is useful to supply written information to the study subject in any case. The most important points that should be addressed are presented in Table 4.

73.6.4.2. Obtaining informed consent and enrolment

The purpose of informed consent is to make sure that persons participate in a study purely on voluntary basis. Participants should not feel obliged to take part based on (material or immaterial) dependence to the investigator. Also they should be informed of all details concerning the study and their participation, and be convinced that no information is withheld from them. They should have the opportunity to ask questions and let additional information influence their decision to take part. The informed consent form states that even after taking the decision to participate and signing the form, the participants may decide to discontinue participation without the obligation to explain to the investigator about this.
Table 4: Issues to be included in information provided to the study population.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose</td>
<td>The purpose of the study should be clearly stated.</td>
</tr>
<tr>
<td>Parameters</td>
<td>The parameters that will be investigated should be specified and the relationship of these parameters with the goal of the study should be clarified.</td>
</tr>
<tr>
<td>Role of participants</td>
<td>The role of the participants should be made clear, specifying in detail when and how samples will be collected, how and when questionnaires will be completed and what other requests are made to the participants. It should be made clear that participation is on a voluntary basis.</td>
</tr>
<tr>
<td>Privacy</td>
<td>Clarify how the privacy of the participants will be protected during and after the study. Will the data collection be anonymous and who will keep the biological samples and the data and for what period of time? Also restrictions and procedures for future use of biological samples or data (by third parties) should be described.</td>
</tr>
<tr>
<td>Recruitment</td>
<td>The procedure for recruitment of participants should be explained (e.g. the use of inclusion/exclusion criteria) and the informed consent procedure should be explained.</td>
</tr>
<tr>
<td>Results</td>
<td>The moment and the way of presenting the study outcome to the participants should be made clear. It is important to inform the participants of any plans to disseminate the information to health authorities or to the scientific community or by uploading the data into a specific computer database.</td>
</tr>
<tr>
<td>Compensation</td>
<td>It should be made clear to what extent participants will be compensated for efforts and expenses</td>
</tr>
<tr>
<td>Financial support</td>
<td>It should be stated how the study is financially supported and which parties are involved.</td>
</tr>
<tr>
<td>Contact</td>
<td>Names and contact information of the study coordinator(s)</td>
</tr>
</tbody>
</table>

**73.6.5. Collection, transport and storage**

In this section the collection, transport and storage of blood, urine, and exhaled air samples will be discussed in general terms. More specific information can be found in biological applications datasheets that have been made available on the internet for specific carcinogenic substances (BIOMONECS, 2006).
73.6.5.1. Blood

Blood can be collected from the fore arm by vena puncture. If only 5-10 μl of blood is required a skin prick with a sterile lancet to collect blood from a vein in the finger of the study person may suffice. For metals special tubes with low metal content are needed. If plasma and blood cells are to be used an anticoagulant should be added. If using an anticoagulant the tubes should be thoroughly mixed directly after blood collection.

If isolated red or white blood cells are needed, it is recommended to precipitate the cells by centrifuge and rinse the cells with a sterile solution of 0.9 % sodium chloride within 8 h after collection. Alternatively, lysis may be required terminate any biochemical activity in the blood cells prior to storage. Isolated cell fractions and plasma may be stored at -20°C to +4°C, depending on the stability of specific analytes. For blood cells to be used in biological assays the addition of freezing medium with stabiliser and freezing at -80 °C is common.

73.6.5.2. Urine

With a spot urine sample an aliquot of urine is collected from one void, e.g. a pre-shift and post-shift sample in workers. A sample collected just after awakening is a well-defined concentrated pre-shift sample.

For post-shift urine collection the study persons should take off any contaminated (work) clothes and wash their hands before collecting a urine sample to avoid contamination of the urine sample. For 24 h urine all urine should be collected. As an alternative the study subject can be asked to collect a part of each void (e.g. midstream), but in that case the total volume of each void should also be registered.

Collection of 24 h samples is not recommended, unless the study persons are well instructed and collection of such samples is needed because of the study purpose. This may be required after an accidental high exposure or in a study where volunteers are exposed under controlled conditions, e.g. to study excretion kinetics.

Pre-labelled polypropylene and polyethylene bottles can be used with a wide opening that can be sealed with a screw cap. When analyzing metals it is generally recommended to rinse the urine container with a 3 % nitric acid solution prior to use. Urine bottles should be filled to a maximum of 3/4 of the container volume when storing at temperatures below 0°C. In spot urine samples, adjustment for the sample density is recommended. The most common correction is by determination of the urinary creatinine content. A normal creatinine is around 1 g/L. Values as low as 0.05 g/L or higher than 2.5 g/l should be treated as an outlier. In that case the creatinine level is not reliable and may be indicative of an error during sample collection or an abnormal kidney function.

Microorganisms may appear in urine samples due to contamination from the ambient environment or due to an infection of the bladder or urethra. Samples may be stored at -18°C. If the samples
are stored at 4 °C for not much longer than 24 h no preservation is needed. Organic preservatives such as natrium azide or citric acid may interfere with the analytical procedure and therefore it is usefulness to verify any interferences with the analysis in advance. Inorganic preservatives that may be used are acid solutions (analytical grade solutions of hydrochloric acid, nitric acid or sulphuric acid) until a pH of approx. 2 is reached. Use of a 6 M solution of hydrochloric acid is effective as a preservative for storage at 4 °C. A warning text should be printed on the label if a preservative is added to the urine bottle in advance. After storage at 4 °C or lower a precipitate may appear, consisting of salts and sometimes protein sediment that could capture some of the analyte inside. It is recommended to gradually warm up the sample to 37°C in a water bath, and homogenise the sample thoroughly before analysing.

**73.6.5.3. Exhaled air**

End-exhaled air is recommended as a well defined fraction that can easily be collected in the field. For collecting an end-exhaled sample a breath sampler can be used (Figure 2). The study person should be instructed to take the sample after a standardized period of time following the end of exposure. The sample should be collected in a clean environment. Breath sampler and solid sorbent tubes may be reused after cleaning. When cleaning, instructions of the supplier of these materials should be followed and materials should be tested before use, to avoid carry over of organic analytes from one sample to the other. Storage of breath samples in the gas phase is not recommended because analytes may be lost due to leakage of the breath sample container, adsorption of the analyte to the sample container and/or by chemical reactions of the analytes with other components in the breath sample. It is recommended to transfer the organic substances from the breath sampler to a solid sorbent within a few hours after sample collection. Once the organic analytes are transferred to a solid sorbent, the sample can be stored at room temperature in a clean environment for a prolonged period of time. It is recommended to seal the sorbent tubes in a gas tight plastic bag to avoid contamination during transportation and storage.

**73.6.6. Chemical analysis**

**73.6.6.1. Performance Characteristics**

The most important characteristics are precision and sensitivity. Precision is expressed in reproducibility and repeatability. Reproducibility is usually expressed as a relative standard deviation (RSD). The criterion for acceptable reproducibility can be taken from a consensus publication from IUPAC (Pocklington, 1990) as the between laboratories reproducibility relative standard deviation (RSDR) that can be calculated from $2^{(1-0.5 \log C)}$ where c is the concentration expressed as a decimal fraction (Figure 7).
Figure 7: RSD\(_R\) in relation to concentration of nickel (µg/L) in background samples. The dotted line represents the RSD\(_R\) required according to IUPAC (1990) in both panels. The raw data are presented in the upper panel. For 7 samples the RSD\(_R\) exceeds the required value. In the lower panel shows that the aggregated data fulfil the IUPAC requirement.
The within laboratory method performance, expressed as the repeatability relative standard deviation (RSDr) is about one half to two thirds of the RSDR (Pocklington et al. 1990).

The sensitivity of a method is determined by the limits of detection (LOD) and quantification (LOQ). The LOD is defined as the lowest amount of analyte in a sample which can be detected but not quantified. The lowest amount of analyte in a sample which can be quantitatively determined with specified precision and accuracy under the certain experimental conditions is called the limit of quantification (LOQ).

73.6.6.2. External quality assurance

The quality of the analysis should be evaluated using reference standards. For biomarkers only few certified reference materials are available from the Community Bureau of Reference (BCR) in Brussels. Round robins are useful for inter-comparison of lab performance using matrix-mixed standards for different biological materials.

73.6.7. Interpretation of results

73.6.7.1. Background values

Some studies provide background values for biomarkers in the general population such as for metals by Brune and co-workers (2003). In 2005 background values were established as part of a EU-project (Table 5). For this campaign samples were collected in 2003 and 2004 in Belgium, Denmark, Germany, Sweden, The Netherlands and the United Kingdom in male and female (1:1) mostly white collar workers, 18-65 of age. All subjects were non-smokers or smokers who stopped smoking more than 6 months prior to sample collection.

73.6.7.2. Biological limit values

The EU has a system of biological limit values, although not many BLVs have been published so far. The ACGIH has a system of Biological Exposure Indices (BEIs) that is updated each year (ACGIH, 2008). In addition BAT-values established by the Deutsche Forschungsgemeinschaft (2008) can also be used.

73.6.7.3. Dissemination of results

It is preferred to collaborate with a physician, who is authorised to keep the study outcome and the key to the person code list (two-way verification of person identity). In this way it is possible for the physician to disclose the data to an individual subject if needed. This may be done if the outcome is unexpected and may be indicative of a risk to the health of the subject.
Table 6: Background values of non-smoking adults (BIOMONECS, 2006)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Biomarker</th>
<th>N</th>
<th>0.95 Percentile</th>
<th>µg/L&lt;sup&gt;a&lt;/sup&gt;</th>
<th>µg/g creatinine&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>Aniline in urine</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.1</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>Arsenic in urine</td>
<td>64</td>
<td>20.4</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>Benzene in end-exhaled air</td>
<td>65</td>
<td>8 ng/L</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S-phenyl-mercapturic acid in urine</td>
<td>64</td>
<td>7.3</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t,t-muconic acid in urine</td>
<td>64</td>
<td>0.32</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Butylbenzylphthalate (BBP)</td>
<td>MBP&lt;sup&gt;c&lt;/sup&gt; in urine</td>
<td>64</td>
<td>116</td>
<td>146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MBzP&lt;sup&gt;d&lt;/sup&gt; in urine</td>
<td>64</td>
<td>29.7</td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cadmium in urine</td>
<td>64</td>
<td>0.36</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>Chloroform in end-exhaled air</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1 ng/L</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>Chromium in urine</td>
<td>64</td>
<td>0.39</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Diethylhexylphthalate (DEHP)</td>
<td>5-oxo-MEHP&lt;sup&gt;e&lt;/sup&gt; in urine</td>
<td>64</td>
<td>82.6</td>
<td>72.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MEHP&lt;sup&gt;e&lt;/sup&gt; in urine</td>
<td>64</td>
<td>25.1</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-hydroxy-MEHP&lt;sup&gt;e&lt;/sup&gt; in urine</td>
<td>64</td>
<td>99.6</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-carboxy-MEHP&lt;sup&gt;e&lt;/sup&gt; in urine</td>
<td>64</td>
<td>142</td>
<td>152</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>Nickel in urine</td>
<td>64</td>
<td>3.3</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Platinum</td>
<td>Platinum in urine</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30 ng/L</td>
<td>35 ng/g</td>
<td></td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>1-hydroxypyrene in urine</td>
<td>64</td>
<td>0.20</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>Tetrachloroethylene in end-exhaled air</td>
<td>54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2 ng/L</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>o-Toluidine</td>
<td>o-toluidine in urine</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Trichloroethylene in end-exhaled air</td>
<td>54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1 ng/L</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Unless otherwise indicated; <sup>b</sup>No subjects from United Kingdom; <sup>c</sup>MBP = mono-n-butyl; <sup>d</sup>MBzP = mono-benzyl phthalate; <sup>e</sup>MEHP = mono-(2-ethylhexyl) phthalate
ACKNOWLEDGEMENTS

Most of the contents of this chapter is based on the results of the project Biological Monitoring of Exposure to Carcinogenic Substances (BIOMONECS) that was supported by the European Union (contract no. QLK4-CT-2002-71801). The author is indebted to dr. L.E. Knudsen, M. Törnqvist, and dr. S. Fustinoni for valuable discussion and comments.

REFERENCES

ACGIH (2008) TLVs and BEIs based on documentation of the Threshold limit values for chemical substances and physical agents & biological exposure indices. ACGIH, Cincinnati.

Battershill, J.M., Burnett, K. and Bull, S. (2008) Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies. Mutagenesis, [ahead of print]


NHANES (National Health and Nutrition Examination Survey)  


FURTHER READING


