GUIDING PRINCIPLES FOR THE USE OF BIOLOGICAL MARKERS IN THE ASSESSMENT OF HUMAN EXPOSURE TO ENVIRONMENTAL FACTORS – AN INTEGRATIVE APPROACH OF EPIDEMIOLOGY AND TOXICOLOGY

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Report on a WHO Consultation

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ABSTRACT

Using biological markers can potentially improve the way in which exposure to environmental factors is assessed. At present, however, only a few valid biological markers are available that can be effectively used in epidemiological studies and the assessment of risk. Future application of biological markers requires collaboration between toxicologists and epidemiologists. To establish guiding principles in this field, the Bilthoven division of the WHO European Centre for Environment and Health organized a consultation. The participants critically reviewed existing methods for the use of biological markers in assessing exposure, and discussed the justification and criteria for biomarker use, the limitations of biomarker-related assessments, confounder problems, aspects of study design, validation needs and ethical issues. Recommendations were made covering each of the topics discussed, and it is hoped that they will serve as guiding principles.

Keywords

BIOLOGICAL MARKERS
ENVIRONMENTAL MONITORING
ENVIRONMENTAL EXPOSURE
ENVIRONMENTAL POLLUTION
EUROPE

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INTRODUCTION

The possible impact of chemical pollution of the environment on human health has created public anxiety worldwide and attracted great attention among regulatory agencies. Consequently, action to prevent and control chemical hazards has been taken in most countries of the WHO European Region. The regulation of new chemicals has been enforced and relies almost completely on toxicological studies performed before marketing. Adequate toxicity data are indispensable for the evaluation of existing chemicals and their effect on the environment, but information from studies of laboratory animals cannot predict with complete certainty the final outcome for humans of exposure to environmental factors. The use of additional toxicological information on a chemical already present in the human environment is therefore of paramount importance. Such information is usually obtained from epidemiological studies.

In principle, epidemiological studies should provide background for the ultimate risk assessment of environmental factors. However, for this to be the case, proper assessment of exposure must be an integral part of such studies. Exposure assessment has proved to be a major difficulty in environmental epidemiology. Much epidemiological research is retrospective, and exposure reconstruction is difficult due to the lack of adequate information on the exposure history of subjects. In most epidemiological studies conducted so far, exposure assessments have relied on measurements of environmental concentrations of a given chemical combined with knowledge of the presence of the study subjects in these environments (residential or occupational history, lifestyle habits, etc.). To discuss the issues related to these methods of exposure assessment, a WHO consultation was held in Stockholm from 14 to 15 August 1993.

Biological markers, or biomarkers, have the potential to improve methods for assessing exposure to environmental factors. These techniques are therefore essential for risk assessment. Biological markers permit the estimation of the biologically effective dose arising from exposure through different

environmental media. They also provide the possibility of a more sensitive assessment of (suspected) effects. Moreover, they allow for an earlier recognition of health outcomes with long latency periods (e.g. carcinogenesis). In addition, biological markers may help in detecting interindividual variations in response to exposure to environmental factors and in identifying population groups at special risk. Finally, they may be useful in clarifying the mechanism(s) by which environmental factors exert adverse effects on health and thus form the basis of effective priority-setting and preventive strategies.

The field of biological markers is at an early stage of development. Only a few valid biological markers are available for epidemiological purposes, both in terms of assessing population exposure and of quantifying risk assessment. Bearing these limitations in mind, it is obvious that the future application of biological markers requires that toxicologists and epidemiologists take an integrative approach. It is necessary to identify the needs for further development of available methods and to agree on criteria for the validation of biological markers in terms of the relation of the measured parameter to the actual level of environmental exposure. Validation of the methods is particularly important with larger population studies.

The purpose of the Consultation on Guiding Principles for the Use of Biological Markers in the Assessment of Human Exposure to Environmental Factors was to bring together toxicologists and epidemiologists to discuss existing biomarker-based methods of assessing exposure to carcinogens and non-carcinogens in terms of their validity, their applicability to exposure assessment and/or risk evaluation, as well as the feasibility of their application in studies of the population in the general environment.

The Consultation was organized by the Bilthoven division of the WHO European Centre for Environment and Health, with financial support from the Netherlands "Stichting Fondsenwervingsacties Volksgezondheid", and hosted by the Department of Epidemiology and Preventive Medicine, University Medical School, Cracow. It was attended by experts from nine countries and WHO staff. Professor K. Hemminki was elected

Chairperson and Dr J.C. Larsen Rapporteur. The working papers and participants are listed in Annexes 1 and 2, respectively. The working papers of the Consultation will be published in a special issue of *Toxicology*.

In addition to reviewing existing methods, the objective of the Consultation was to establish a set of guiding principles covering the justification and criteria for biomarker use, the limitations of biomarker-related assessments, confounder problems, aspects of study design, validation needs and ethical issues.

Particular emphasis was placed on:

- DNA and protein adducts;
- levels of environmental pollutants and/or their stable metabolites in tissues and body fluids;
- early biochemical markers of effects, for example enzyme induction, oncogene activation, markers of oxidative damage;
- biokinetic and stability aspects of biomarkers;
- validity aspects of studies using biomarkers (relation to environmental exposure);
- application of biomarkers in epidemiological studies.

DISCUSSION

The objective of biological monitoring of exposure is to determine the internal dose or, ideally, the biologically effective dose (or target dose). This then provides a basis for assessing the health risks of a chemical. This assessment relies on the measurement of markers in different biological media such as blood, alveolar air, urine or tissues. These biomarkers may consist of the concentration of the parent compound or its metabolites, in a nonadverse biological effect related to the internal dose or else of the amount of chemical that covalently interacts with target molecules (adducts). They can be defined as biologically measurable indicators of the internal dose which can be used as an independent (or possibly confounding) variable in the study of associations between exposure and adverse

effects. Apart from biomarkers of exposure, there are also biomarkers of effect and of susceptibility. The Consultation focused mainly on biomarkers of exposure.

DNA and protein adducts

Many carcinogenic chemicals cause DNA damage through adduct formation in target and non-target tissue. Adducts are also formed in RNA and proteins and they can be used as surrogate measures of DNA binding. Methods for determining DNA adducts include the 32P-postlabelling assay, immunoassay and synchronous fluorescence spectroscopy. Repair of DNA adducts is considered an important process, and a person's DNA repair capability may be more important than the metabolic activation in determining that person's susceptibility. The urinary output of DNA adducts is a measure of DNA repair. Additionally, methods are available for detecting excreted urinary RNA and DNA adducts. The protein adduct techniques include both immunological and chemical assays. These techniques have been applied in occupational and environmental studies. Quantitative aspects of many of the assays have not been resolved, and there are interlaboratory differences in the results obtained. Furthermore, different assays do not necessarily measure the same types of adducts. Relationships between adduct levels in the surrogate tissues and target organs are poorly established. This is likely to reflect the activity of xenobiotic metabolism and DNA repair in various tissues. The relationship of adducts to disease outcome has not been established.

Early biochemical markers of effects

Among the enzyme systems involved in metabolic activation of environmental chemicals to proximate or ultimate carcinogens, the cytochrome P450 (CYP) superfamily is the most important, in particular the CYP1A family (polycyclic aromatic hydrocarbons (PAHs), aromatic amines), the CYP2E1 family (nitrosamines, halogenated hydrocarbons) and the CYP3A family (mycotoxins, PAHs). Genetic polymorphisms of the important carcinogen-activating CYPs have not been clearly identified. The activities

appear unimodally distributed and individual activities are easily modified by environmental factors. Cytochromes of the P450 family are biomarkers of susceptibility. They may serve as biomarkers of effect if certain related enzymes are induced or if gene mutation takes place due to a certain exposure. CYP activities can be measured in humans by different non-invasive methods, requiring only blood or urine samples. Several of the enzymes have been associated with risk of various cancers without relation to any particular carcinogen.

There is growing evidence suggesting that neoplasia in many cases may require changes in at least two classes of cellular genes: proto-oncogenes which become activated and tumour-suppressor genes which become inactivated. Proto-oncogenes may be activated to oncogenes, the protein products of which are important for cellular growth and differentiation. The mechanisms by which such genes may be involved in carcinogenesis may involve single point mutations, gene amplifications or chromosomal translocations. Oncogene activation and tumour suppressor gene inactivation may emerge as markers of early changes in malignant transformation.

Living organisms are continuously exposed to reactive oxygen species as a consequence of biochemical reactions and external factors. The rate of oxidative DNA modifications in humans is very high and extensive repair is necessary, yet the steady state of oxidatively modified bases in human DNA is about 25 per 10^5 bases. The relatively simple determination of 8-oxodeoxyguanosine (8-oxodG) in DNA from target or accessible surrogate tissues by means of HPLC with electrochemical detections probably represents or correlates with the most important oxidative DNA damage. Urinary excretion of 8-oxodG appears to be an attractive candidate for a non-invasive biomarker of oxidative DNA modifications. Several lines of evidence support the importance of oxidative modifications in aging and degenerative changes, e.g. cancer, and constitutively and environmentally induced oxidative stress may be a considerable modifier of the individual cancer risk.

Levels of environmental pollutants and/or their stable metabolites in tissues and body fluids

The assessment of human exposure to toxic trace elements can be performed by examining suitable biological specimens. The human materials that are accessible for sampling include blood and urine, but hair and nails may also be used. Successful attempts have also been made to measure accumulation of lead and non-metal inorganic pollutants, such as fluorine in deciduous teeth, to demonstrate non-occupational exposure to these elements in children. Hair samples are simpler to collect, transport and store than samples of blood and urine. While blood and urine concentrations reflect recent exposure within a relatively wide range, hair as well as nails reflects long or past exposure. The data provided by human hair analysis may well serve as a basis for identifying population groups at specific risk due to excessive environmental contamination, as well as reflect the benefits of interventions and protective measures. So far, human hair has proved a valuable specimen for the assessments of exposure to arsenic, mercury and selenium. For other metals, more validation is needed in order to use human hair analysis for assessing exposure.

Levels of organic pollutants and/or their metabolites in body tissues or fluids are specific markers of internal dose and, provided their toxicokinetic and dynamic properties are known, they may also be used as predictors of effects. However, most organic compounds are readily metabolized and excreted from the human body, and measurements of their concentrations in human tissue and urine can only be used in prospective environmental health studies. In addition, the predictive value of assessing exposure depends on proper timing and frequency of sampling according to the toxicokinetic properties of the compound. The use of levels of organic compounds and/or metabolites in blood, urine or exhaled air for assessing exposure has a long tradition in occupational studies.

Levels of persistent organic compounds, such as dioxins and polychlorinated biphenyls (PCBs), are useful as markers of exposure in retrospective studies, too, as their tissue levels mainly reflect previous exposures. From knowledge of their biological half-lives,

actual past exposures can be estimated with a certain confidence. Recent determination of blood concentrations of dioxins and related compounds in blood samples from individuals that in earlier epidemiological studies were estimated as heavily exposed have demonstrated the weakness of exposure assessments that rely on measurements of environmental concentrations of the chemical combined with theoretical calculations from knowledge of residential or occupational history, lifestyle habits, etc.

Biokinetic and stability aspects of biomarkers

Biological monitoring relies primarily on the knowledge of the toxicokinetics of chemicals. The kinetic aspects determine whether the test is likely to reflect recent exposure or to integrate exposure over a certain period. The parameter that best summarizes the behaviour of a chemical in a biological system is the elimination half-life, which reflects both the affinity of the chemical for the biological matrix and the efficiency of excretory or metabolic processes. The half-life to be considered for a given test is determined mainly by the time of sampling. If the sample is taken during exposure or immediately after, the half-life of application is that reflecting the elimination from the central compartments, while when the sample is taken several days or weeks after the exposure, the relevant half-life is that corresponding to the elimination from deeper compartments from which the chemical is cleared more slowly. Since chemically induced diseases usually develop following repeated exposure over many years, the information needed is whether the biomarker reflects the exposure over a short time before sampling or whether it can integrate exposure over a period sufficiently long to be related to the development of disease.

Biomarkers of exposure can be classified into four main categories.

- *Biomarkers with a half-life less than 12 hours.* The concentrations of solvents in alveolar air or in blood are representatives of this category.
- *Biomarkers with a half-life between 12 and 100 hours.* This category includes many solvents or metabolites that

have a slow component (such as body fat) in their elimination. The use of 1-hydroxypyrene as a biomarker of PAH exposure also belongs to this category.

- *Biomarkers with a half-life between 100 hours and 6 months.* This category includes adducts formed with DNA and blood or plasma concentrations of cumulative toxins such as heavy metals.
- *Biomarkers with a half-life more than 6 months.* These biomarkers reflect the elimination of cumulative chemicals from their storage sites. Examples are cadmium in kidneys, lead in bones, and dioxins and PCBs in serum or adipose fat. They can be used in epidemiological studies for a quantitative assessment of cumulative exposure.

When a biomarker is used for assessing exposure in epidemiological studies it is very important to consider the stability of that marker during transportation and storage. The factors most likely to affect the stability of the marker are evaporation, chemical deterioration, precipitation, adsorption on vessel surfaces and contamination.

Validity aspects of studies using biomarkers (relation to environmental exposure)

Biomarkers may prove useful in increasing the precision of exposure estimates during field epidemiological studies of environmental exposures. The determination of the validity of exposure biomarkers is, however, a laborious process. It is also a process that necessitates collaboration between laboratory and field scientists if biological markers of exposure are to be useful tools in environmental epidemiology. Valid biomarkers of exposure will be those dose markers that have biological relevance, defined pharmacokinetics, temporal relevance and defined background variability.

Biological relevance (content validity).

If an exposure marker has biological relevance it will represent part of a chain of events that are a subset of exposure opportunities and a pool from which outcome events are likely to occur. Thus a DNA adduct formed from exposure to PAHs will have content validity if it represents the interaction of PAHs and DNA. The validity of a marker of exposure will depend on its relationship to both exposure and outcome. This relationship is difficult to prove in studies on humans. At the current level of understanding, discussions of group risks rather than individual risk are warranted.

Defined pharmacokinetics.

Knowledge of pharmacokinetics is important in determining the frequency and timing of sampling and the tissues or fluids most appropriate for study. It also guides the interpretation of data on dose and effect obtained in a target tissue or a surrogate.

Temporal relevance.

Appropriate sampling of biological specimens requires that the temporal relationships of markers to external exposure or to a disease be understood. Whether a marker reflects recent or cumulative exposures, peaks or averages, depends on the pharmacokinetics of the chemical and its persistence in the biological sample. Most measures of internal dose reflect recent exposures. Exceptions are substances that are fat soluble and are stored in adipose tissue. Haemoglobin is a good integrating dosimeter over the four-month life span of the erythrocytes. In contrast, human serum albumin has a half-life of 20–25 days. The kinetics of markers such as carcinogen-DNA adducts is complicated by there being apparently two compartments for adducts in DNA. The majority of adducts are rapidly lost due to repair while other adducts reside in a compartment that is longer lived and consistent with cell turnover.

Defined background variability

It is important to know the range of values of a given marker in a "normal" population. Pristine populations are rare, so even non-

exposed populations are generally subject to some exposure. These amounts can vary greatly. However, within a given study the normal range needs to be known to allow for the interpretation of abnormalities. In addition, interindividual variation and intra-individual variation are important contributors to noise or background in monitoring or epidemiological studies and should be characterized before large-scale applications of a particular biomarker.

Further, confounding variables should be accounted for in studies that use biological markers. Such factors include age, sex, race, cigarette smoking, alcohol consumption, diet, drug use, genetic factors, and pre-existing health problems. Assay variability also tends to cloud potential association.

Application of biomarkers in epidemiological studies

For a biomarker of exposure to be of use in epidemiological studies, there is a need for data which show that levels of biomarkers firmly correlate with different quantitative levels of external exposure. Further, it should be demonstrated that a single assessment of biomarkers reflects a prevailing long-term exposure as commonly investigated in environmental epidemiology. In the absence of such evidence current studies have to make careful attempts to account for other (non-environmental) factors that influence an individual's level of biomarkers. There is only sparse information available as to what extent exposures in the past can be reflected by today's assessment of biomarkers for that exposure or an associated early biological effect.

The aim of using biomarkers of exposure in cancer epidemiology is to permit a quantitative assessment of risk that is more accurate than an assessment using external exposure measures. Employing biomarkers will also permit identification of populations or individuals at a stage in the carcinogenic process when intervention can prevent or cure the cancer. In this regard the marker will serve as a dependent variable, indicative of a biological exposure outcome with a predictable association with cancer.

Before markers can be applied in such a fashion, however, the associations between the exposure, marker and cancer incidence must be determined. Once markers have been validated as representing true biological responses to specific exposures, it is necessary to determine the quantitative relationships between exposure, marker expression and cancer risk. These investigations will require the use of epidemiological methods to examine the fraction of cancer attributable to biological pathways involving the marker and the degree to which the marker mediates the carcinogenic response to exposure. The traditional epidemiological measures of attributable proportion and adjusted relative risk can be used to obtain this information. The measures can be readily derived from both case control and cohort studies. However, a number of methodological problems must still be addressed, such as devising approaches that can distinguish between different mechanistic pathways between the exposure and marker of interest, determining important factors that are truly confounders, and applying methods that can incorporate information on variables such as marker half-life, pharmacokinetics, cell turnover and repair mechanisms.

Health risk assessments based on biomarkers of exposure should be compared to assessments based on external exposure, and both should apply to the same environmentally exposed population. Further, both predictions should be checked against the real outcome. To predict "local" effects external exposure is nearly always preferable. Biomarkers compared to external exposure may theoretically better predict systemic effects, but the reduction to an environmental origin will often be less accurate.

Application of biomarkers in population studies of non-malignant diseases

Characterization of exposure by means of biomarkers is important in defining the relationships between exposure and non-malignant disease. Detection of acute effects after exposure may not always be paralleled by the sudden increase of a marker (in the blood or urine). This becomes particularly true for a target organ such as the lung, which may show an immediate response following acute exposure,

before a change in some biochemical or biological indicator becomes apparent.

The situation may be less complex when long-term exposure is considered in relation to a chronic disease. Since chronic diseases are often based on multifactorial pathogenetic mechanisms (e.g. asthma, chronic bronchitis, ischaemic heart disease), detection of biomarkers should be able to characterize changes related to a constant long-term exposure (e.g. 20–30 years of cigarette smoking, living for a long period in an urban area).

Individual predisposition should also be determined. In fact, well defined factors may determine the risk of developing some chronic diseases, e.g. atopy or bronchial hyperreactivity for chronic obstructive lung disease. Exposure in vulnerable subjects may therefore be even more significant. Moreover, it is important to ascertain whether long-term exposure per se or in association with other factors may cause the development of the disease, and biomarkers may be useful in this respect. The use of biomarkers in large population studies would allow better assessment of long-term exposure and increase understanding of relationships between various factors and in turn their relationship to health outcomes.

Ethical issues

There are a variety of ethical issues involved in the use of biological markers in human studies. Some of these include: Can specimens collected for one purpose be used for future purposes? How should biomarker assay and study results be interpreted and communicated to subjects? Who should have access to biomarker information? What role should psychological harm be considered when recruiting subjects into biomarker studies?

The current level of uncertainty related to the contribution to risk for most markers should be strongly emphasized, because many researchers are using these markers in intervention studies as if the relations were clearly understood.

CONCLUSIONS

1. In addition to traditional methods for assessing environmental exposure, biomarkers can provide information on a number of issues.

   (a) Detection of toxic metals in the sampled human materials could help to determine multimedia integrative exposure to toxic metals and metalloids.

   (b) In retrospective studies levels of persistent organic compounds are useful as markers of exposure since their tissue levels mainly reflect previous cumulative exposures.

   (c) Well validated biomarkers of exposure can be useful in assessing the effectiveness of interventions, e.g. in the case of blood lead levels or similar markers that do not cumulate in the matrix studied.

2. Although historical data are still useful in environmental studies, more reliable exposure measures than combination of environmental levels and such estimators as residential history, job titles, lifestyle habits and individual perceptions are highly desirable.

3. A rational application of biomarkers of exposure in epidemiological studies or in any other situation is possible only when information is available on the toxicokinetics/toxicodynamics and the stability of the measured parameter, and its significance as an index of the recent or past exposure has been determined.

4. In non-neoplastic disease, where the use of a biomarker appears more suitable to evaluate chronic effects, it is particularly important to understand the temporal relationships with exposure.

5. Notwithstanding the need for validation studies, biomarkers should preferentially be applied in prospective studies with stored biological samples. The analysis of stored samples from cases and controls within a cohort (nested case–control study) presents an elegant epidemiological method. However, there also needs to be additional evidence that long-term storage of samples does not hamper the measurement of the biological markers.

6. Caution should be taken when emphasizing that traditional exposure measures are preferable to biomarkers when both are equally associated to risk, because to date, well controlled, statistically powerful studies of the relation of markers to the risk of various diseases, in particular cancer, have not been conducted.

7. Results can be interpreted on a group or on an individual basis. The individual interpretation can be envisaged only for confirmed results and when one can refer to quantitative relations between the internal dose and external exposure or the risk of toxic effects. Unfortunately, the latter information is available for only a very limited number of markers of exposure. For the vast majority of biomarkers currently under development (e.g. adducts with DNA or protein) only a group-basis interpretation is feasible.

8. Minor variation in the sensitivity or specificity of the marker assay can strongly bias the observed risk when the prevalence of the marker is high (sensitivity effect) or low (specificity effect).

9. Information is needed on how susceptibility may change the effects of exposure to environmental chemicals. In order to strengthen the power of epidemiological studies biomarkers of susceptibility should be included, preferentially chosen according to importance for the activation of chemicals.

10. A distinction should be made between specific and non-specific biomarkers. Non-specific biomarkers may be of particular importance in assessing effects from multiple exposures, optionally supplemented with selective biomarkers.

11. Validation needs to be conducted at both the laboratory and population levels. Assessing population validity involves determining variability in subgroups of the population. These studies require the use of epidemiological methods.

RECOMMENDATIONS

The following recommendations serve as a set of guiding principles for the use of biomarkers.

1. The most useful markers for population studies are those that integrate the dose received by the organism, or still better by the target organ(s), over a toxicologically relevant period of exposure. Such markers exist for heavy metals (e.g. cadmium in urine or lead in bones). They can also be developed for non-cumulative chemicals capable of forming adducts with long half-lived macromolecules. The use of these markers in epidemiology in conjunction with classical methods of exposure assessment should be encouraged because they represent reliable independent variables for the study of dose–response relations.

2. Epidemiological application of biomarkers can be useful in understanding mechanisms of non-neoplastic disease, how they relate to risk factors, and so on. Different epidemiological studies may be proposed; for example, cross-sectional studies comparing different environmental situations or nested case-control studies. Prospective studies may give information on feasibility with respect to predicting disease. Additional work on study design and analysis methods is needed to improve

information on competing pathways between exposure and disease.

3. Epidemiological studies on pollutant-induced disease should use markers capable of detecting early toxic effects on target organs (early markers of disease).

4. In choosing between parameters of external exposure (EE) versus biomarkers of exposure (BM) to predict the health risk of a specific part of the population to environmental exposures at least the following aspects should considered:

   - effects on superficial tissues usually requires EE;
   - presence of susceptible subgroups due to toxicokinetics or behaviour requires BM;
   - predictive validity of each (depending on the elaboration and the time elapsed between the exposure and the study;
   - availability of EE or BM to response (effect) relationships (or specific health-based allowable limit values);
   - expected cooperation and psychological reaction of the population (after being informed);
   - costs.

5. There should be a rationale for using markers instead of classic exposure assessment methods.

6. Owing to the high analytical chemical sensitivity required in most cases and the complexity of environmental exposures, measurement of the tissue levels of pollutants and/or the metabolites as biomarkers of exposure demands the incorporation of quality control procedures. In order to allow comparisons between different studies to be made, interlaboratory standardizations should be conducted.

7. There is a need to determine ways to incorporate other information relevant to the formation of markers, kinetics and dynamics, and to identify the role of possible confounders or

effect modifiers within the context of the exposure→marker→ disease paradigm.

8. Markers with short half-lives reflecting very recent exposure must be used with caution in population studies. These markers may lead to a misclassification of subjects with equivalent integrated doses and thereby distort the analysis of dose–response–effect relations. They must always be used in combination with other indices of past exposure, including at least the duration of exposure. An integrated assessment of the cumulative exposure using the latter can be attempted only if data from a repeated sampling are available. In the absence of such data, the main interest of these markers is to provide confirmatory evidence of the exposure which cannot be extrapolated over time. These markers may be useful, however, in identifying unsuspected confounders or improving the adjustment for known confounders (e.g. tobacco smoking).

9. The limitation of the use of biomarkers in media (blood or urine) which do not reflect events at target organs must be considered. Thus, it is important to develop biomarkers that can characterize what is happening at target organs.

10. Investigators should obtain information on confounders, such as demographic and behavioural factors, and collateral information, such as historical exposure. The same is true for susceptibility factors, if appropriate.

11. In case of exposure to complex mixtures it is advisable to use a range of markers including specific and non-specific indicators of exposure, since the inclusion of more than one biomarker can increase sensitivity and strengthen their predictive power in epidemiological studies.

12. Owing to the small number of subjects, the statistical power of most studies conducted until now is not large enough. Errors in the measurement of exposure or biomarkers should always be

considered, and the design of the study should include explicit consideration of the statistical power.

13. The choice of population is a key issue in the assessment of dose–response relationship. A representative sample may not always be the optimal solution in this respect. An alternative approach may be considered, such as choosing a sufficiently large group of subjects with distinct exposures.

14. In order to overcome the current problems in using biomarkers, validation studies are required with the following features.

    - Epidemiological studies with thorough control for confounding are individual-based (cohort or case control studies). Consequently, validation studies on biomarkers need to be individual-based, too.
    - Quantitative individual-based data on total external exposure should be related to quantitative measurements of biomarker status. In this approach confounding variables might also be assessed through biomarkers. External exposure should be assessed by special monitoring or sampling systems.
    - Repeated sampling of biomarkers is essential to establish whether a single measurement could be representative of long-term prevailing exposures. Such studies need to be carried out urgently and should be, if possible, supplemented with parallel assessment of external exposure for the purpose of correlation, as mentioned above.
    - To the extent possible it should be clarified whether current biomarkers can validly reflect external exposure in the past. For a validation study of this type external exposure measurements in the past at an individual level should be correlated to individual-based biomarker measurements at present.

15. In order to establish the relationship between exposure and adduct level, further development of specific adduct tests are needed. This can be done with the help of standard compounds, which also allow quantitation in the assays. An international bank of standard compounds usable in the postlabelling assay would be a major advance in human biomonitoring. Specific DNA adducts should be assayed together with specific protein adducts in the same studies.

16. Epidemiological studies using biomarkers of exposure should focus on the relationships between the internal dose and effects at a later stage in order to assess the health significance and predictive value of the marker.

17. Investigators should recognize the variety of ethical issues involved in the use of biological markers.

Annex 1

WORKING PAPERS[a]

ICP/PCS 204/6	Validity criteria for the use of biological markers of exposure to chemical agents in environmental epidemiology, by P. Schulte
ICP/PCS 204/7	DNA and protein adducts, by K. Hemminki.
ICP/PCS 204/8	Early biochemical markers of effects: enzyme induction, oncogene activation and markers of oxidative damage, by H. Poulsen
ICP/PCS 204/9	Use of human hair as biomarker in the assessment of exposure to pollutants in occupational and environmental settings, by V. Bencko
ICP/PCS 204/10	Levels of pollutants and their metabolites – exposures to organic substances, by J.C. Larsen
ICP/PCS 204/11	Biokinetics and stability aspects of biomarkers. recommendations for application in population studies, by A. Bernard
ICP/PCS 204/12	Application of biomarkers in population studies for respiratory non-malignant diseases, by P. Paoletti
ICP/PCS 204/13	Application of biological markers in cancer epidemiology, by B. Trock
ICP/PCS 204/14	Design of studies for validation of biomarkers of exposure and their effective use in environmental epidemiology, by J. Wahrendorf

[a] Copies can be obtained from the WHO European Centre on Environment and Health, Bilthoven, Netherlands.

ICP/PCS 204/15 Biomarkers of exposure versus parameters of external exposure; practical applications in estimating health risks, by M. Verberk

ICP/PCS 204/16 Application of biomarkers in heavily polluted industrialized areas of countries of central and eastern Europe, by G. Motykiewicz

ICP/PCS 204/17 Environmental determinants of CYP1A1 Induction in placental tissue in populations with different ambient air pollution exposures. The Cracow Study, by W. Jedrychowski

Annex 2

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