Guidance for the Interpretation of Biomonitoring Data

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CONTENTS

SUMMARY

Measuring the levels of chemical substances in human body fluids and tissues has been in routine use in industry and parts of the wider public health community for more than 50 years. The increased availability of analytical technologies with constantly decreasing detection limits has made biomonitoring techniques both more accessible and more sensitive. However, although human biomonitoring samples may be comparatively easy to obtain and analyse, their interpretation is often difficult.

Biomonitoring has many benefits, most notably in its ability to evaluate human exposures to chemicals and its potential to improve the accuracy of related health risk assessments. For any valid health risk assessment a basic knowledge and understanding of the underlying dose-effect relationships is essential. Unfortunately, this information is often lacking. Even when it is available, several other factors need to be taken into account for a valid interpretation. This report sets out the considerations that need to be applied in order that available biomonitoring data can be reliably interpreted within the context of their associated uncertainties.

There are a number of considerations that enable the relevance of any biological monitoring result to be interpreted reliably. If biomonitoring information is to be used to evaluate and describe health risks, information is required on 4 key elements: a sample's analytical integrity; the extent to which toxicokinetic considerations have been accounted for; the relevance of the data for health effects; and how the data align with other available information. This document describes the level of understanding that is necessary for each element and identifies how the application of data varies according to the level of understanding, including the relative importance of each element. The elements are then incorporated into a framework, built upon established scientific criteria, that enables any data to be evaluated with respect to the portion of the risk assessment process in which it can be reliably applied. In addition, the report identifies a number of related issues concerning the ethics of taking samples and to the communication of findings that require further discussion.

The report, by nature, is intended both to offer a considered view of the available science and to serve as a catalyst for stimulating discussion on some of the broader issues presented by the application of biomonitoring technologies today. As such, ECETOC considers that the contents of this document should not only be applied, but also be used to further the discussion on the steps necessary for the better use and application of biomonitoring data.

1. INTRODUCTION

Measuring the levels of substances, their metabolites or their adducts in human body fluids and tissues is not new. The technique has been in routine use within industry and the wider public health community for over 50 years. Historically, biological monitoring has been used within a comparatively clear framework for assessing and managing associated health risks e.g. comparison of the findings with health-based action levels or to monitor/identify defined exposure trends within a population (e.g. blood lead levels amongst occupationally-exposed groups; populations supplied with water via lead piping; or those living adjacent to heavily trafficked roads).

Over recent years the wider availability of improved analytical technologies has made biomonitoring techniques more accessible. In addition, changes in the analytical specificity and sensitivity of biomonitoring techniques, often by orders of magnitude, have allowed the detection of more materials at much lower concentrations. These developments have many potential benefits, most notably in the ability to consistently apply biomonitoring as a tool for the widespread evaluation of exposures and finally for health risk assessments. However, together with these benefits, some drawbacks have also become apparent. The most evident of these is the tendency to interpret (often inadvertently) biomonitoring findings beyond the boundaries within which the biomarker can be applied reliably. This is of particular significance now that personalised exposure information from biomonitoring has also become of interest at the individual as well as societal levels by requests for sampling and analysis when adverse effects are merely suspected from environmental exposure (Weis *et al*, 2005).

Technological progress is often accompanied by certain drawbacks. This is also true for the developments in biomonitoring. Indeed, much can be learned from examining the accumulated experiences arising from the past use of biomonitoring in the occupational and public health settings. One example (HMSO, 1972) illustrates this well:

A new method of biomonitoring for occupational lead exposures was developed. It presented a number of advantages over the historic method for blood collection and analysis and offered equivalent, if not improved, analytical accuracy. Because of these advantages (reduced analytical cost; ease of sample collection and analysis), the method was implemented at a major UK lead production facility.

The introduction of the new method immediately identified a seemingly major issue; that previous blood levels were several times below the values identified by the new method. An accusation was made that this was the result of a 'cover up' by the company. The UK government of the time initiated an independent committee of enquiry. The committee established that the new method was valid *per se,* but:

- Inadequate quality control procedures accompanied its introduction;
- inadequate quality assurance was available to ensure analytical accuracy and consistency;
- if the findings had been better communicated it would have allayed unnecessary fears;
- there was a lack of transparency in the process that, in turn, generated mistrust.

When account was made of all the above elements, the new method yielded equivalent results to the historical values.

Recognising a large number of developments in the availability and use of biomonitoring data over the past decade and recognising that such trends are likely to continue, ECETOC established a Task Force with the following terms of reference:

- Develop criteria that will facilitate the consistent use of biomonitoring data;
- develop a framework and guidance on the interpretation of human biomonitoring data for risk assessment and risk management purpose;
- test the proposed framework using a number of case studies covering a range of human exposures;
- present the conclusions of the Task Force to external workshops;
- serve as a network of scientists to track and provide input to ongoing developments in the field of biomonitoring.

In establishing this activity, ECETOC was conscious that there was a need to initiate a discussion amongst the broader stakeholder community concerning how such an objective might be achieved. ECETOC constituted a Task Force, consisting of representatives of ECETOC member

companies, academia and other organisations, with the aim of identifying a potential solution to the core challenge, the merits of which would be debated within relevant scientific and regulatory fora. This process has involved dialogue with European institutions and international organisations.

The resultant document represents the consensus of these various inputs and is, by nature, intended both to offer a considered view of the available science and to serve as a catalyst for stimulating discussion on some of the broader issues presented by the increasing application of biomonitoring technologies in the public health fields. The document does not deal in detail with the ethical considerations that need to be addressed when undertaking any form of human biomonitoring. Indeed, the lack of a formalised framework that enables ethical considerations to be accounted for within the context of how any biomonitoring study (especially those involving the general public) is conducted would appear to be an issue that requires wider discussion amongst the scientific and public health communities (see Section 6, Conclusions and recommendations).

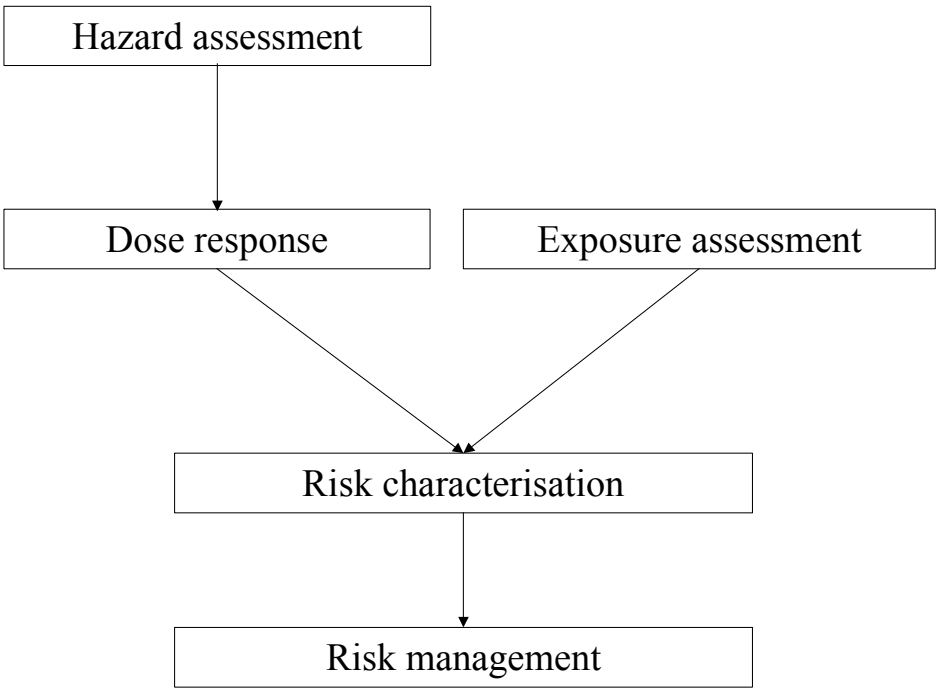
2. DEFINITIONS

During the course of the preparation of this document, it became apparent that in order to facilitate a more consistent use of biomonitoring data, an agreement was needed on the context in which the data were to be used as well on the definitions themselves.

Risk assessment and management

Given that different views exist on what constitutes the elements of a process for risk assessment and management, the group adopted the definitions agreed jointly by the International Programme on Chemical Safety (WHO/IPCS) and the Organisation for Economic Cooperation and Development (OECD) (IPCS, 2003) as the basis for its discussions. Consistent with these definitions, the process for risk assessment and management adopted is shown in Figure 1.

Figure 1: Process for risk assessment and management (adapted from EC, 2003)

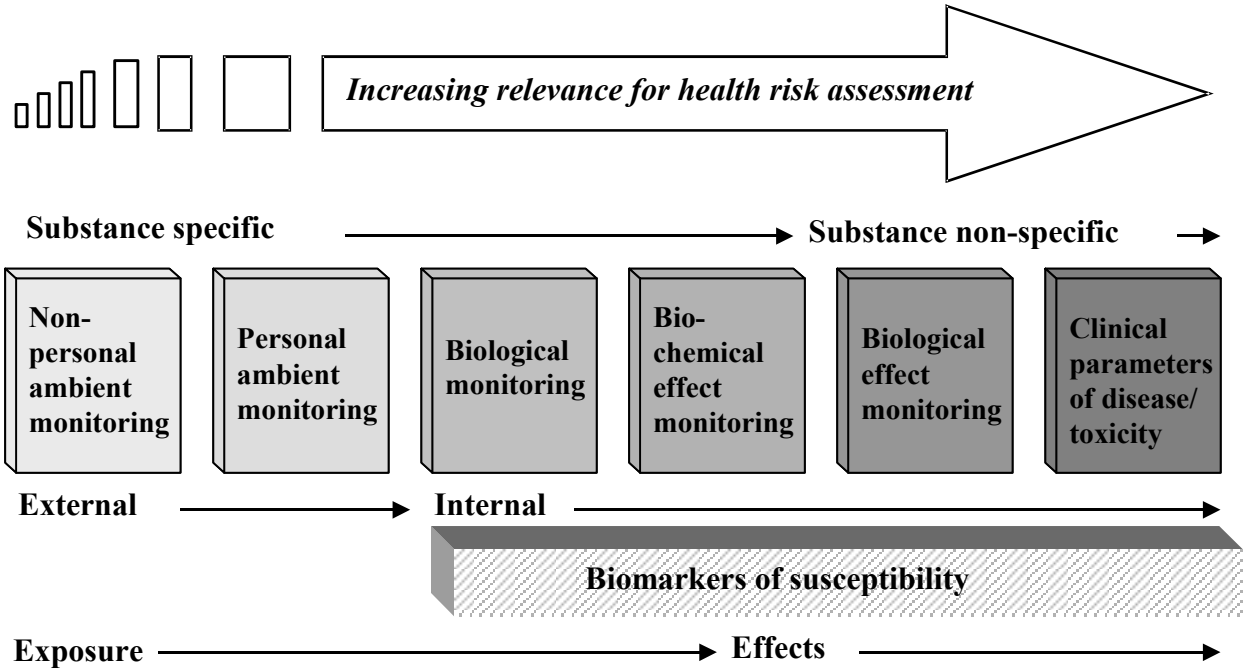


Biomarkers

The terms biomonitoring and biomarkers are increasingly being used, but their use is often confusing since the terms are applied for different concepts and different situations, varying from

occupational exposure monitoring to environmental pollution monitoring and from genotyping to clinical measurements. This report is limited to the use of human biomonitoring, where biomonitoring is a part of the exposure-disease continuum as depicted in Figure 2, along with other monitoring tools such as (personal) ambient monitoring.

Figure 2: Monitoring techniques as part of the exposure-disease continuum



Non-personal external (ambient) monitoring includes, amongst others, static air monitoring, monitoring of soil, drinking-, ground or surface water monitoring, and 'food basket' monitoring.
Personal external monitoring includes, amongst others, personal air monitoring and dermal exposure monitoring.

Biomonitoring is understood in the current report to be a general term comprising the following subcategories:

1. biological monitoring – biomarkers of exposure (also: internal dose or body burden)
2. biochemical effect monitoring – biomarkers of effective dose (also: tissue dose)
3. a. biological effect monitoring – biomarkers of effect
   b. clinical parameters – biomarkers of disease

Both *biological monitoring* and *biochemical effect monitoring* are crucial methods to help better understand the complex relationships between external and internal exposure and, consequently, the potential adverse health effects that may result from exposure. Just like ambient monitoring, both *biological monitoring* and *biochemical effect monitoring* should be regarded as *exposure monitoring* methods with high specificity for the substance that is being measured. In fact, in

biological monitoring as well as in biochemical effect monitoring, each individual serves as his or her own dose monitor. Both methods give a measure of the total, actual exposure regardless of the route of exposure. While biological monitoring simply reflects total exposure and is applicable to most substances, biochemical effect monitoring reflects the effective dose of a chemical, usually a potentially genotoxic substance. It does this by measuring a substances reaction product with an endogenous macromolecule indicating that the chemical was not only bioactive but also was absorbed into the body and reached the tissues (hence: tissue dose). In some cases, it is possible to measure reaction products in the ultimate target tissue. In these instances the concentration may be referred to as *target tissue dose*. The concept of *target tissue dose*, however, is applicable not only to the biomonitoring of genotoxic compounds but may also be used for other compounds with a specific target tissue (e.g. cadmium in the kidney). Typical examples of *biological monitoring* are:

- the determination of metals (e.g. mercury, arsenic or lead) in blood or urine;
- the determination of unchanged substances (e.g. dioxins, PCBs or benzene) in adipose tissue, milk, urine, or blood;
- the determination of specific metabolites of a chemical (e.g. S-phenylmercapturic acid as metabolite of benzene) in urine;
- the determination of volatile compounds (unchanged substances or metabolites) in exhaled breath.

Typical examples of *biochemical effect monitoring* include the determination of adducts of a specific chemical (e.g. ethylene oxide or polycyclic aromatic hydrocarbons) to DNA or a protein, such as albumin or haemoglobin. However, it should be realised that biochemical effect monitoring solely provides evidence for interaction of a chemical with the body and cannot be interpreted in terms of health risk assessment without additional data on dose-response relationships. Some forms of biochemical effect monitoring, such as the determination of haemoglobin adducts, have additional benefits since they allow time-integrated exposure monitoring over long periods of time (up to several months) at a point in time when intervention may still be possible.

Biological effect monitoring measures early and, in most cases, reversible biological effects, which do not necessarily lead to health effects whereas *abnormal clinical parameters* are an expression of the (beginning of) disease. In general, in biological effect monitoring natural phenomena are measured for which a ‘normal’ background value may be established. This may be influenced by various physiological and environmental factors that are not related to chemical exposure. This renders most methods of biological effect monitoring intrinsically non-specific.

Typical examples of *biological effect monitoring* include measurements such as cholinesterase activity in blood, zinc protoporphyrin, sister chromatid exchanges and other chromosomal

aberrations, and microproteinuria. Various parameters assessed in biological effect monitoring may also be used in clinical practice and form a seamless continuum as a more or less arbitrarily set value may distinguish a minor biological effect from an effect that is considered clinically relevant. For example albuminuria may be used in biological effect monitoring (microalbuminuria) as a biomarker of early renal function effects but it is also used as a *clinical parameter* for instance to assess renal function impairment in diabetics.

Biomarkers are potentially influenced by various factors, such as lifestyle and physiology, including genotype and phenotype. *Biomarkers of susceptibility* refer to the genotype or phenotype of an organism/individual and are indicators of an inherent or acquired ability of it to respond to the challenge of exposure to a specific substance, for instance through the expression of certain isoforms of cytochrome P450, glutathione transferases or *N*-acetyltransferases. In addition, factors such as iron-status, nutritional status etc. may also be regarded as biomarkers of susceptibility. These biomarkers differ from the other biomarkers in that they reflect potential inter-individual differences in uptake and metabolism of chemicals and, consequently, potential differences in health risks. Biomarkers of susceptibility may, like lifestyle factors such as smoking and drinking behaviour, explain differences in biomarker results between individuals with identical exposure profiles (DFG, 2004).

The accuracy of the exposure determination as well as the relevance for health increases from non-personal ambient monitoring, via personal ambient monitoring, to biological monitoring and biochemical effect monitoring. The relevance for health increases further with biological effect monitoring and clinical effect monitoring, but with a loss of specificity with regard to the chemical (or physical) factor associated with the health effect. In general, for health risk assessments, biological effect monitoring and biochemical effect monitoring provide the best and most reliable information both in terms of exposure and potential health effects related to certain exposures (see Figure 2).

The characteristics of biomonitoring to evaluate internal exposure and early biochemical and biological effects make biomonitoring a potentially more accurate risk assessment tool than external monitoring such as air monitoring and surface contamination monitoring. Unfortunately, however, the number of substances for which a validated biomonitoring method is available is limited compared to those for which environmental monitoring methods are on hand.

Probably the most fundamental principle of risk assessment is that adverse health effects are related to dose, i.e. to levels and/or duration of exposure. In the domain of occupational biomonitoring, biological limit values have been established for a number of substances. Usually, these limit values are based on a correlation between the biomonitoring values and personal air monitoring data. These are, in turn, related to an understanding or estimation of the level of exposure associated with adverse health effects. These adverse health effects may be based either

on actual data obtained from human exposure or, more and more frequently, on extrapolation from data obtained in animal experiments. By contrast, only a limited number of health-based biomarker limit values have been established for the general population. Some of these include the reference and guidance values for heavy metals and persistent organic pollutants established by the German Human Biomonitoring Commission (Ewers *et al*, 1999; Wilhelm *et al*, 2003, 2004).

Some of the challenges associated with the use of non-occupational biomonitoring data reside in the interpretation in terms of adverse health effects in a non-homogeneous population exposed at levels that are usually several orders of magnitude lower than the no observed adverse effect levels (NOAELs) in animal experiments.

3. PURPOSE AND USE OF BIOMONITORING (BOTH EXPOSURE AND EFFECTS MONITORING)

3.1 Availability of biomonitoring data

A wide and increasing amount of biomonitoring data is becoming available. Historically, biomonitoring has mostly been used in the occupational settings, i.e. as a survey tool and also a research tool to study uptake and metabolism of chemicals and associated health risks mainly related to high exposure situations such as accidental contaminations. However, due to recent technical developments biomonitoring is increasingly used to study environmental exposures. This type of biomonitoring often builds on experience from occupational settings.

Nowadays many governments around the world have established environmental biomonitoring programmes that report representative values of selected biomonitoring values in non-occupationally exposed populations. These programmes complement a range of other monitoring programmes that study levels of chemicals in the environment such as in drinking water, food, fish and other biota and air.

The current status of biomonitoring in Europe has recently been described in the baseline report of the technical working group for biomonitoring of children established as part of the European Environment and Health Strategy (COM, 2003). In this report about 100 biomonitoring activities in children are identified and evaluated. These include initiatives such as the German Environmental Survey (GerES, 2005), the German Environmental Survey on Children (Pesch *et al*, 2002; LGA, 2005), the biomonitoring programme in humans on environmental health which is conducted on behalf of the Flemish Ministries of Environment and Health (WVC, 2005), the Polish biomonitoring programmes (Indulski *et al*, 1999; Heinrich-Ramm *et al*, 2000; Jakubowski and Trzcinka-Ochocka, 2005), the French biomonitoring programme (Huel *et al*, 2002), and the Portuguese biomonitoring programmes. They also include breast milk nutrition, and research activities, e.g. the Concerted Actions in the Fifth EU Framework Programme: ChildrenGenoNetwork (2005), PINCHE (2005), AIRNET (2005) and a number of research programmes in the Fifth EU Framework Programme such as Plutocracy (2005), MENDOS (2005), and BIOMONECS (2005).

In the US, the third National Report on Human Exposure to Environmental Chemicals has recently been published (US CDC, 2005) providing part of an ongoing assessment of the exposure of the US population to selected environmental chemicals using biomonitoring. The report provides data for 148 chemicals, including metals, polycyclic aromatic hydrocarbons, dioxins and dioxin-like substances, polychlorinated biphenyls, phthalates, phyto-oestrogens, organochlorine and organophosphate pesticides, and pyrethroids.

This report was based on measurements taken from selected participants in the National Health and Nutrition Examination Survey (NHANES) which is undertaken serially to collect data on the health and nutrition status of the US population. Trends in the levels of chemicals are reported. Results are interpreted with reference to the ranges of concentrations found in the population studied. In some instances, results were also interpreted against reference values.

This report adds to other US biomonitoring data compilations such as the National Human Adipose Tissue Survey (collected from 1970 to 1987) and the National Human Exposure Assessment Survey (NHEXAS) carried out over the period 1980-1990 to provide multi-pathway, multi-media population exposure to certain chemical classes.

The Arctic Monitoring and Assessment Programme of the Arctic Council continues to add significantly to studies of substances in the Arctic environment to enable assessments of levels in vulnerable geographic regions and ethnic populations. This work is supported by the governments of Canada, Denmark, Finland, Iceland, Norway, Sweden, USA, and the Russian Federation (www.arctic-council.org).

In addition to the above reported studies, reports from other countries are found in the scientific literature focusing on selected public health issues such as environmental exposure to lead in Australia. Targeted initiatives by other governments examining blood, urine and umbilical cord measurements are also underway.

In respect of existing information it is important to differentiate between research projects and survey projects. Research projects aim at the generation or the improvement of knowledge on the causal links between environmental factors and health by hypothesis generation and testing. Survey projects aim at periodic measurements to produce information on the prevalence of exposure to environmental agents (Knudsen, 2004). Survey projects may be used in the public health arena with a view to developing and evaluating policies designed to protect the health of the general population.

Another differentiation that should be made is between occupational and environmental monitoring since the purpose of these activities may differ with regard to ethical and legal issues.

3.2 Uses of biomonitoring data

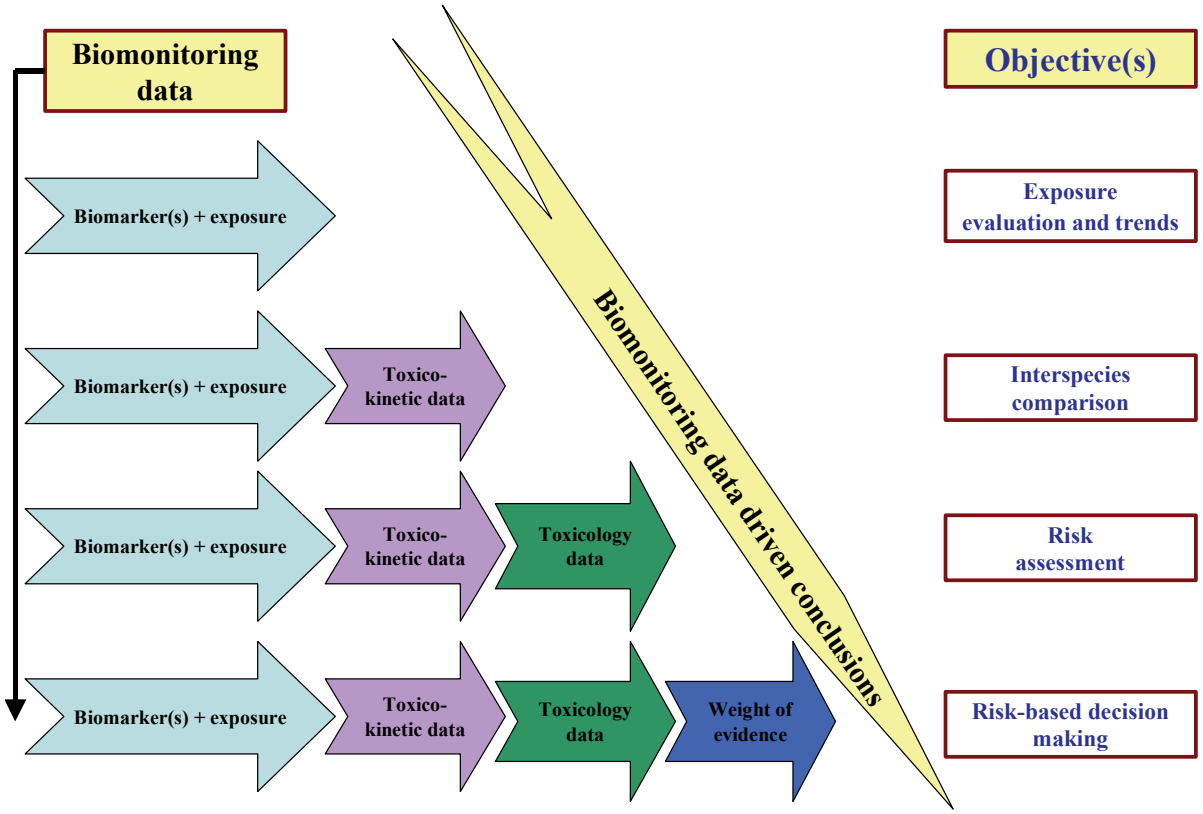
The availability of existing information and ongoing studies shows that biomonitoring can contribute to a number of different goals:

1. Biomonitoring can improve occupational and environmental exposure assessments since it may provide a more relevant estimate of exposure than e.g. ambient monitoring. Substances may enter the body through various exposure pathways. By measuring in the human body itself, biomarkers of exposure integrate all sources and routes of exposure, take into account toxicokinetics and give more precise information on the effective dose.
2. Biomonitoring can be used to follow up the ultimate fate of environmental substances into the human body. Human uptake may be predicted based on mathematical modelling of behaviour of substances in the environment and on animal experiments. This may imply uncertainties related to dispersion of substances through the environment, and uncertainties related to uptake and distribution of substances. Human biomonitoring may validate this knowledge and may improve the accuracy and predictive power of the models. Biomonitoring may provide insight into the contribution of different environmental compartments (e.g. air, water, food) and emission sources to body burden when these external sources can be related to the internal exposure levels.
3. If used in surveillance programmes, biomonitoring may produce information on the concentration of substances in different populations. Sensitive and vulnerable groups may be identified (high exposure or high response). Spatial and temporal differences in population exposure may be documented. Biomonitoring may allow an evaluation of the effectiveness of measures to reduce emissions in terms of impact on human exposure (survey projects, see below).
4. Biomonitoring may provide early warning signals, which indicate the need for further follow up in terms of exposure or in terms of health effects. Exposure markers (e.g. in the workplace) may indicate whether preventive measures have been adequate or whether additional measures are needed. In carefully designed studies, which include potential confounding factors, effect markers may be considered as early warning signals integrating the effects of substances with the same mode of action.
5. Biomonitoring may improve knowledge on the link between environmental factors and health (research projects, see below). Although not providing information on health effects *a priori*, exposure and effect biomarkers are additional indicators within the 'source emissions-environmental concentrations-exposure-biological effects-health effects' chain. Exposure and effect biomarkers are much closer to health effects than environmental monitoring (see Figure 2).
6. To establish reference ranges that can be used by physicians and scientists to determine whether or not a person or group of people has an unusually high exposure.

4. GUIDANCE ON INTERPRETATION

Several aspects need to be taken into account when evaluating reports that incorporate or use biomonitoring data. The analytical method(s) used to detect the biomarker should be relatively standardised methodology that can be used by any competent laboratory. Where possible, some type of internal standardisation should be included. In addition, inter-laboratory comparison of analyses should be considered to help secure consistency. The biomarker being measured also needs to be evaluated in terms of its representation of exposure. If the goal is to evaluate exposure or exposure trends then these considerations, coupled with an understanding of the source(s) of exposure, should be sufficient for drawing conclusions about exposure based on biological monitoring or biochemical effect monitoring data. However, if there is the need to make inter-species comparisons, develop a risk assessment, or make risk-based decisions, additional toxicology and toxicokinetic data are needed. Furthermore, human exposure or health data related to the chemical of interest can, if available, provide additional valuable information for decision-making.

Figure 3: Decision-making when using biomonitoring data



It should be noted that Figure 3 depicts an idealistic situation, where data are gathered in a logical order. In practice, however, it will often happen that toxicological data (hazard data), usually based on animal studies, are available without any detailed knowledge on the toxicokinetics in either animals or humans. In such cases, interpretation of the results is limited to the following of exposure trends.

4.1 Analytical integrity

Although some biomarker measurements are carried out according to internationally accepted standards, a large number are not carried out by standardised methodology. In many cases these analytical approaches have been developed 'in house' according to local laboratory requirements, and interlaboratory comparisons have not been done. When such comparisons have been made, large variations between laboratories have, in some cases, been revealed (e.g. 8-oxodG (Collins *et al*, 2004), ^{32}P-postlabelling (Phillips and Castegnaro, 1999), N-(2-hydroxyethyl)-valine (Törnqvist *et al*, 1992)). These variations were found to be due to different methods of analysis (such as immunological versus chromatographic assays, gas chromatography versus high performance liquid chromatography and electrochemical versus mass spectrometric detection), different methods of calibration, and different methods of standardisation. This indicates the need for detailed validation of such approaches.

Internal quality control and, if possible, adherence of the participating laboratories with national or international quality standards are strongly recommended. For a biomonitoring procedure to be accepted as validated, a number of parameters are to be considered, including:

- A standard operating procedure should be used for the entire study, including the pre-analytical phase (sample collection, sample storage), the analytical phase (analytical procedures, quality controls), as well as the post-analytical phase (statistical analyses of the data, reporting of the results).
- Conditions for sample collection and storage should be standardised. The stability of the biomarker in the biological fluid should be known, both immediately after collection and for (long-term) storage under the conditions used. It is often helpful to include biological samples 'spiked' with known concentrations of the biomarker in the collection procedure to account for losses during transport and storage.
- For certain types of biomonitoring, such as environmental biomonitoring of the general population, ethical clearance and permission must be obtained prior to sample collection.
- Recoveries, reproducibility and accuracy should be determined (and be in acceptable range(s)) for analytical methods used.
- If possible, a pure reference sample of the material being analysed should be available, for calibration purposes. Additionally, availability of a sample of the biological tissue that is

being used for the analysis, containing a known amount of the analyte or modification, is very valuable for checking that recovery is consistent.
- If the methodology permits it, an internal standard should be added to improve accuracy.
- Control samples and 'blank' analyses should be studied to ensure that there is no contamination or artifactual production of the analyte caused by the sample isolation or analysis (e.g. as can be seen with oxidative DNA damage).
- For a better comparability of urinary data correction for differences in urine concentration using creatinine concentrations should be considered.
- The limit of detection/quantification of the method should be determined and reported.

4.2 Ability to describe exposure (toxicokinetics)

Trace levels of natural or man-made substances may enter the human body through a variety of routes, including eating, breathing, drinking, and contact with the surroundings. Biological monitoring and biochemical effect monitoring provide a snapshot of those substances or their metabolites present in the body independent of the route of exposure and thus, make it possible to draw conclusions on the integrated dose. For differentiating between exposures, the monitoring method must be selective and measure exclusively the object of the biomonitoring programme. This selectivity depends not only on analytical specificity, but also, especially in situations in which the biomarker is not the parent chemical, on other sources of the metabolite. For example, varying amounts of phenol are derived from dietary components and may be excreted in urine. It is therefore hardly possible to estimate exposure to benzene by this analysis, despite the fact that phenol is the main metabolite of benzene. Only at very high occupational exposure levels (> 10 ppm benzene) can urinary concentrations of phenol accurately reflect benzene uptake. At non-occupational or low levels of occupational exposure such measurements are completely useless and misleading. In these cases, other more specific biomarkers like blood benzene or urinary *S*-phenylmercapturic acid are more reliable. It is therefore important to use the appropriate biomarker, taking into account, before starting a biomonitoring programme, the estimated external exposure level. The parameter to be measured should be the one that is optimal under the local conditions.

Another problem is the ability to describe exposure properly if there is endogenous formation of the biomarker within the human body. This is, for instance, the case with macromolecular adducts of ethylene oxide, for biomarkers such as hippuric acid and virtually all biomarkers of effect. An 'endogenous' contribution to the biomonitoring result may make the data hard to interpret in terms of external chemical exposure. Under these circumstances a detailed investigation of the endogenous processes may be required. The most widely used biomarkers of exposure derive from the measurement of parent compounds and/or their metabolites in blood or urine. For genotoxic substances, the consensus view is that biological monitoring of parent compounds or

their metabolites in blood, urine or other bodily fluids is preferred for monitoring short-term exposures. Biochemical effect monitoring (such as the determination of haemoglobin adducts) is preferred for measuring the cumulative internal dose due to repeated exposures to this type of substance. This is because most macromolecular adducts have a significantly longer apparent half-life than the genotoxic substances themselves or their metabolites. In addition to these kinetic-based considerations, measurements of adducts formed by the reaction of electrophilic metabolites of genotoxic compounds with macromolecules are especially useful because they represent the dose that has escaped the detoxification process and has reached protein or DNA. Methods to quantify such adducts were originally developed by Ehrenberg's group (Ehrenberg, 1984), in Stockholm in order to determine what was referred to as the 'target' or true dose following an exposure to a genotoxic compound and this methodology has been successfully applied over the past decades (Törnqvist *et al*, 2002; Boogaard, 2002).

Not only is the choice of a suitable biomarker essential, but the disposition kinetics of the chemical are also important with respect to the possible biological variability relative to external chemical exposure. Thus, biological monitoring is normally not feasible for chemicals with a half-life of less than 2 hours. Biological monitoring of occupational exposures to chemicals with half-lives ranging from 2 to 10 hours requires a sample taken at the end of a working day that represents the integrated exposure during the working day. In the case of longer half-lives (up to about 100 hour) the optimal time for sampling is at the end of the 5-day working week and the determinations reflect exposure during the preceding few days. For substances and their metabolites with even longer half-lives and for biochemical effect monitoring using stable adducts, the biomonitoring result is independent of the time of sampling. In such cases kinetic considerations of the sampling strategy are less important. The same applies to most non-occupational situations where the balance of substance uptake and excretion usually results in an apparent steady-state situation.

To be able to relate biomonitoring data back to exposure, a detailed knowledge of human metabolism and toxicokinetics is desirable if not essential. For chemicals with a long history of occupational monitoring this may be available. However, it is more likely that an extrapolation from laboratory studies must be used. This may present some difficulties because of large inter-species variations in metabolism as well as the higher doses used in animal studies compared to human exposure. It may therefore be necessary, in some instances, to carry out controlled human metabolism and kinetic studies at very low levels of exposure. There are two principal reasons for doing this (Wilks *et al,* 1993): firstly, to identify metabolic pathways and thus target metabolites, which can subsequently be used in biological monitoring studies at the workplace or in the general population. Secondly, to compare biomonitoring data with toxicology information gained from laboratory studies. This allows extrapolation back from the excretion of a metabolite to exposure to the parent compound, i.e. to understand the human toxicokinetics of the compound. It is of course necessary for such studies to undergo rigorous ethical and scientific scrutiny and to

comply with all relevant national and international regulations and guidelines for human studies (Wilks and Minton, 1999).

4.3 Ability to relate to effects

The evaluation of the adverse effects of a substance (hazard evaluation) is usually based on human experience (e.g. resulting from accidental exposure or epidemiology), if available, as well as toxicological data from animal experiments. Acute and long-term toxicity, including genotoxicity, carcinogenicity and reproductive toxicity can be evaluated with established toxicological methods. Data on dose-response relationships and on the modes of action are of high relevance for the overall risk assessment. The dose without an observed adverse effect (e.g. the NOAEL) or the benchmark dose are important starting points for a quantitative risk assessment. Information on toxicokinetics and metabolism are useful tools for species comparisons and for external exposure and internal dose extrapolations.

Biomarker studies should be hypothesis driven (ILSI/HESI, 2004). The hypothesis of a causal relationship between biomarkers of exposure and observed effects needs to be set in advance, prior to the initiation of the study. The hypothesis under study should be based on an understanding of the mode of action of a chemical, on animal data, or on comparative epidemiological studies. Appropriate, pre-defined statistical methods need to be applied to evaluate the significance of the correlations between biomarkers of exposure and observed effects. Validity needs to be considered by evaluating analytical integrity, the relevance, specificity and sensitivity (IPCS, 2001).

Important aspects for the ability to relate biomarker concentrations to effects are the knowledge of background levels in the general population, the relation between external and internal exposure as well as the relation between biomarker concentration and the total dose, the estimation of the inter- and intra-individual variability as well as the evaluation of confounding factors (systematic errors) that can affect the marker. It is helpful to employ criteria classically used to establish causality, for example those proposed by Bradford-Hill (1965) and further developed by Vineis and Porta (1996). Overall validity needs to be considered by evaluating analytical integrity, the relevance, specificity and sensitivity for example by applying the framework developed by IPCS (2002). Other considerations necessary for any such framework are discussed elsewhere (ECETOC, 2004).

4.4 Overall evaluation, weight of evidence

Evaluation of causal relationships between an exposure and a specific effect is very complex. It often involves integrating data from many studies that differ in terms of experimental conditions and the parameters examined. This is termed the 'weight of evidence' approach and used in different areas of evaluation (e.g. Harvey and Johnson 2002, US FDA 2001, Jacobs *et al* 2003).

The weight of evidence approach is the evaluation of all available data on a specific compound or hypothesis and has to be done on a case-by-case basis. Data that need to be considered are the available (animal) toxicological and/or human epidemiological data, as well as exposure information. It also takes into account structure activity relationship (SAR) considerations and a plausibility check, e.g. with the evaluation of cross-species comparability.

Consistency and biological plausibility of data or a specific hypothesis should be checked. With regard to consistency, disparate findings in the literature may not only be due to differences in study design but may also indicate that there are factors other than the substance under study involved. Thus the evidence either in favour of, or against, a particular hypothesis has to be considered weak and requiring further study. The evaluation of the mode of action for a substance of concern is part of the biological plausibility check. Of special importance for the extrapolation of the observed effect to, for instance, low exposure levels is to clarify whether there is a threshold or a non-threshold mode of action. For the plausibility check of a hypothesis the comparison of the dose at which the suspect agent is thought to induce adverse health effects with the assumed human exposure is important. For example, if animal data show a clear no effect dose for agents that are thought to have a threshold mode of action, and a toxic effect at very high doses only, it is unlikely that the toxic effect would occur in humans at doses orders of magnitude below the no observed effect level in animals.

If, based on the weight of evidence, a hypothesis for a causal link of a biomarker of exposure and an assumed effect is not confirmed, the next step is to evaluate alternative hypotheses. For example, the examination of confounding factors might lead to follow-up hypotheses.

4.4.1 Proposed framework for use of biomonitoring data

The framework proposed by the Task Force (Table 1) builds upon the above four principal considerations by setting out the required knowledge and information needed for the main applications of biomonitoring in risk assessment, notably:

- to establish exposure trends;
- to characterise exposures;

- to investigate linkages between exposure and adverse health effects; and
- to facilitate risk management and standard setting.

The framework has been tested against a number of illustrative case studies (see Appendix B) covering a variety of biomonitoring situations, including parabens, perfluorooctane sulfonate, hexachlorobutadiene, DDT, aflatoxin, and data from the WWF biomonitoring survey as well as biomonitoring results from occupational and environmental health studies.

Table 1: Proposed framework for the evaluation of biomonitoring data

Purpose of study	**Required knowledge**			
	Analytical integrity	**Toxicokinetics**	**Health effects**	**Weight of evidence**
Trends in exposures	×			
Characterisation of exposures	×	×		
Investigation of health impacts	×	×	×	
Risk assessment and standard setting	×	×	×	×

An expanded version of the framework proposed above is given in Appendix A.

5. OTHER CONSIDERATIONS

5.1 Ethics

Ethical considerations arise in biomonitoring because of the need to take human samples of the media of interest, e.g. blood and urine.

In the past, when biomonitoring was carried out mostly in occupational settings or in accordance with prescribed regulatory requirements, ethical issues relating to the confidentiality of information and communication of results were well established. However, in the broader context discussed in this report the ethical issues associated with population biomonitoring are not yet well codified.

In the case where biomonitoring is conducted as part of a research initiative the involvement of an ethics review board is necessary. Currently, the role and need for formal ethical review of public health surveillance activities is under discussion within the WHO. General international guidelines for the ethical review of epidemiological studies published by the Council for International Organizations of Medical Sciences in 1991 are also under review (www.cioms.ch/frame_1991_texts_of_guidelines.htm).

Use of the biomonitoring data collected in any surveillance activity is often constrained by ethical issues relating to confidentiality. For example in the regional assessments conducted by the Arctic Monitoring and Assessment Programme (AMAP, www.amap.no) it was not possible to release or share data that would have enabled statistical comparison between different ethnic groups or regions.

The need to address ethical issues to the fullest extent in the conduct and use of biomonitoring studies is acknowledged by the Task Force. While considered outside its terms of reference it is thought that the above activities and others could provide a focus for necessary work to establish general guidelines for use of biomonitoring information in risk assessment activities.

5.2 Communication of findings

Occupational and environmental exposures are diverse and changing, often necessitating more individual approaches to exposure identification and risk assessment. Biomarkers may provide clues of undesirable exposures or potential adverse health effects and thus can serve as prompts for the need to intervene. Well-defined communication strategies respecting the autonomy of study persons and data protection need to be in place to prevent misinterpretation and misunderstanding of the data.

Stakeholders in the biomonitoring process can include the study participants, employers (for workplace studies), researchers/investigators, as well as the families of the study participants, scientific and medical institutions and society at large. Special information needs must be considered where human samples are used for environmental biomonitoring of the general population, particularly those concerning consent, confidentiality and follow-up. Where the collection of samples and personal information addresses health status and is used for research, this should be preceded by a notification of the project to an appropriate ethical committee. This would include a protocol describing any other risks to the participants, the information (oral or written) given to participants, and the way of obtaining informed consent. The protocol should also include information about follow-up and accessibility of individual information. If a register, (database), with personal information is set up, this register must be approved by the data protection authorities. Guidelines must be available stating who has access and how information can be obtained. Identical considerations must be made for biological material, kept in bio-banks, and potentially available for further analysis.

The information about exposure and susceptibility gained through biological monitoring is personal and can be predictive of health impairments. Such information may therefore be discriminative and thus sensitive in relation to future opportunities in occupation, learning possibilities and health insurance. The information must be kept confidential with precise guidelines on who is allowed to use the information. It is also important, however, to make sure that information about test results that indicate a health impairment that may be treated/prevented is given in a timely manner. This may be problematic if data and sample banks maintain information in an anonymous manner. Instead, the double coding approach currently used within the pharmaceutical industry is recommended.

Where biomonitoring studies provide information of interest to local communities and society, further communications to the public will be necessary. Availability of informative web pages and radio and television broadcasts may serve to improve transparency in the communication process and help ensure that key aspects of the study design, anonymised results, etc. are known and understood by wider groups.

6. CONCLUSIONS AND RECOMMENDATIONS

Biomonitoring data manifest themselves in many forms and are no longer simply measurements of the substance or its primary metabolites, but can now extend to molecular markers of exposure or effect. Biomonitoring data can be extremely useful within the context of the processes of exposure assessment, risk assessment and risk management. Indeed, they provide the only integrated measure of exposure and, it could be argued, the only currently effective means for assessing the significance of dermal and oral exposures.

This document sets out the basis for a rational framework against which the quality of available biomonitoring data might be evaluated with respect to the boundaries within which they are relevant.

In developing the framework, the Task Force identified a number of areas where further actions are recommended:

1. Although the utility of the framework has been tested against a number of different case studies, further validation is required to better establish the boundaries within which it can be reliably applied. Specifically, although biomarkers of exposure and (biochemical) effect have been investigated in some detail, further work should be done to define more clearly how biomarkers of effect (and susceptibility) could be incorporated.
2. A more extensive library of case studies should be developed that would serve as a training tool to help risk assessors etc. understand how different forms of biomonitoring data should be evaluated and what conclusions can or cannot be drawn from them.
3. Guidance should be developed on how study findings should be communicated to different interest groups. The guidance would need to cover the communication of results to individuals and groups, as well as including the wider communication of findings to external audiences.

In developing their views, the Task Force also identified a number of related activities that, although outside the scope of the terms of reference, in their view, require further discussion:

- The rules and considerations that govern the ethics of how biomonitoring surveys and programmes are initiated, managed and maintained should be clarified. Rules that could provide a basis for such guidance exist. But the extent to which biomonitoring surveys presently address these issues is inconsistent. This inconsistency leads to unnecessary confusion by virtue of the different expectations that are raised and which participants believe will be addressed. The development of clear and concise, ethically based guidance would help minimise this.

- Although good guidance is currently available on the considerations that should be made when undertaking biomonitoring (IPCS, 2001), the guidance mostly addresses the theoretical considerations that should accompany the development and execution of studies. There is a lack of clear and succinct guidance on the practical aspects of how and in what context such studies might be undertaken in practice.
- To improve the way in which society is able to understand the significance of biomonitoring data, there is a need to develop health-based biomonitoring limit values for the general population (including, when appropriate, relevant subgroups) for substances of high public health concern. A significant number of such values already exist in the occupational context (e.g. ACGIH, HSE, DFG), but there are only a limited number of examples for non-occupational applications (Ewers *et al,* 1999; Wilhelm *et al*, 2003, 2004). It is suggested that there is a need to ensure that such an activity is overseen by an authoritative body for example in a manner similar to that used to develop air quality guidelines for public health protection.

The ideas for improving the use of available biomonitoring information are commended for wider discussion. It is ECETOC's hope that this will stimulate a science-based debate that will lead to the increased recognition of the utility and value of biomarkers and reduce the frequency with which such indicators are interpreted incorrectly. In conjunction with the need for a framework, there is also a need for an activity that would help identify which particular biomarker is best suited for use in a particular circumstance.

APPENDIX A: SUGGESTED FRAMEWORK FOR INTERPRETING BIOMARKER DATA

Boundary of interpretability	Framework element									
	Analytical integrity			Toxicokinetics			Health effects	Weight of evidence		Case study
	Validated protocol	QA and QC	Adequate sample size	Timing of samples	Knowledge of kinetics	Related knowledge of exposure parameters	Knowledge of mechanism	Shape of dose response curve	Nature of related findings[a]	ECETOC Appendix B example
Exposure trends Provides a snapshot indication of the presence of a substance in a ***group***. Number of samples is a function of data representivity. Data cannot be used in wider interpretation and not related to known health outcomes.	×	×	×							B1, B2, B3, B4, B5
Exposure characterisation Provides an indication of the presence of a substance in a ***group*** and how this may relate to sources of exposure. Number of samples is a function of data representivity. Data can be used to describe exposure sources and routes if key parameters detailed for individuals within the study group.	×	×	×	×	×	×				B6, B7, B8

[a] Nature of related findings refers to the strength and relevance of the biomarker data when viewed in the context of other human and animal evidence

Boundary of interpretability	Framework element									
	Analytical integrity			Toxicokinetics			Health effects	Weight of evidence		Case study
	Validated protocol	QA and QC	Adequate sample size	Timing of samples	Knowledge of kinetics	Related knowledge of exposure parameters	Knowledge of mechanism	Shape of dose response curve	Nature of related findings[a]	ECETOC Appendix B example
Health impact investigation Describes the presence of a substance in a ***group*** and how this plausibly relates to (defined) health effects. Certainty of association is a function of the detailed mechanistic knowledge. Number of samples is a function of data representivity. Data cannot be used to describe individual health outcomes.	×	×	×	×	×	×	×			B9, B10, B11
Risk assessment and standard setting Describes the presence of a substance in a ***group*** and how this relates to both (defined) exposures and (group) health outcomes, including risk. Certainty of association is a function of the detailed mechanistic knowledge. Data cannot be used to describe individual health outcomes although it may be possible to describe elevated risks. Strength of causality determined by weight of evidence.	×	×	×	×	×	×	×	×	×	B12, B13, B14

[a] Nature of related findings refers to the strength and relevance of the biomarker data when viewed in the context of other human and animal evidence

APPENDIX B: EXAMPLES

B.1 Parabens (para-hydroxybenzoates)

B.1.1 Description of the study

The study by Darbre *et al* (2004a) assesses the mean concentration of individual parabens in samples of 20 human breast tumours by high-pressure liquid chromatography followed by tandem mass spectrometry. Parabens were found in all 20 patients with an average concentration of 20.6 ± 4.2 ng/g tissue. Comparison of individual parabens showed that methylparaben was present at the highest level and represented 62% of the total paraben recovered in the extractions. This study demonstrates the presence of parabens in human breast tissue. It was discussed in an editorial (Harvey and Everett 2004), a series of letters to the editor (Golden and Gandy, 2004; Jeffrey and Williams, 2004; Flowers, 2004), replies by the authors (Darbre *et al,* 2004b,c,d) and was evaluated by Scientific Committees (EFSA, 2004; SCCP, 2005).

B.1.2 Assessment of the study

Analytical integrity

The analytical integrity cannot readily be assessed. Sophisticated analytical techniques and statistics were used to calculate the values reported, but no quality controls or standards were used. There are issues with potential contamination since high levels of parabens were detected in blank samples (sometimes higher than in breast tumour tissue) and the variability was high (Golden and Gandy, 2004; Jeffrey and Williams, 2004; Flowers, 2004; EFSA, 2004; SCCP, 2005).

Ability to describe exposure

The data reported are in fact internal exposure data. However, since parabens are used widely as preservatives in cosmetic, food and pharmaceutical products and no specific information on use of these products is reported, the external exposure is unknown. In addition, parabens are natural products (Jeffrey and Williams, 2004). No information on backgrounds of patients was given (Jeffrey and Williams, 2004) nor was there any information on medication (which may have involved exposure to parabens) (Golden and Gandy, 2004; SCCP, 2005).

Ability to relate the measured exposure parameters to effects

The ability to relate the measured exposure parameters to effects is limited. A comprehensive database is available on dose-effect relationships of parabens and parabens are heavily regulated. The toxicity of parabens in general is low (SCCP, 2005). The study did not analyse any control tissue (breast or other tissue) (Golden and Gandy, 2004; EFSA, 2004; Harvey and Everett, 2004; SCCP, 2005). No other chemicals were analysed (Harvey and Everett, 2004).

Weight of evidence that observed effects are related to the exposure

The evidence that paraben concentrations are related to breast cancer is virtually non-existent, let alone a causal relationship. In general, relationships between the use of products with parabens and breast cancer have not been reported (Golden and Gandy, 2004; Jeffrey and Williams, 2004; EFSA, 2004; Harvey and Everett, 2004; SCCP, 2005). In animal studies parabens are not carcinogenic (Jeffrey and Williams, 2004) and, in general, their toxicity is low. The hypothesis of accumulation, as postulated by the authors, is inconsistent with literature and recent data. The least lipophilic paraben (methylparaben) was detected in the highest concentration and it appears that tissue concentrations are in almost reverse order to their partition coefficients (Golden and Gandy, 2004). In addition, accumulation cannot be assessed by a single measurement (Jeffrey and Williams, 2004).

B.1.3 Applicability in risk assessment

Deficits in the study design, in combination with the absence of a relationship between dose and carcinogenic effects and the lack of exposure information, indicate that the data are not suitable to be used in a risk assessment.

B.1.4 Additional comments

No additional comments

B.2 2005 WWF biomonitoring survey

B.2.1 Description of the study

The World Wildlife Fund biomonitoring survey (WWF, 2005) analysed the blood samples of 8 (celebrity) volunteers in the UK. The samples were analysed for 104 unspecified chemicals. For

each personality, the number of identified chemicals was given (but not specifically identified), together with information related to identified chemical groups. No more than 36 substances were detected (with the lower end of the range being 10) in any individual. The study was reported as finding that "All of the celebrities were contaminated with toxic chemicals" and that their blood samples were "without exception […] laden with chemicals that could be harming them".

B.2.2 Assessment of the study

Analytical integrity

The analytical integrity cannot be assessed in any way. Neither the details of the sampling protocols, analytical techniques followed, nor details of the laboratory that was used, were reported. Similarly, no information on quality control considerations was reported, including limits of detection.

Ability to describe exposure

The study provides no useful ability to describe exposure: the study subjects were self selected and no attempt was reported as having been made to obtain exposure histories which might help explain the nature of the results, although the report hypothesises that variations in concentrations between different individuals may have been due to a range of lifestyle factors e.g. diet, emissions from articles.

Ability to relate the measured exposure parameters to effects

The study was not intended to provide any ability to relate the measured exposure parameters to effects. It simply summarises the general findings for the (named) individuals and speculates on what the source of the exposures might be.

Weight of evidence that observed effects are related to the exposure

The report makes reference to a previous WWF report to try to put the findings in context. However, this attempt at a weight of evidence comparison is impossible to perform when (either group or individual) exposure concentrations are unreported. Moreover, comparison with only a single study, when many others exist, is biased.

B.2.3 Applicability in risk assessment

The study has almost no role to play in the risk assessment process. Key information on exposure is lacking. The reported findings of the study that purport to show that all blood samples are "without exception... laden with chemicals that could be harming them" are unsupportable. No data are provided on which substances were analysed, nor on the relevant effect and exposure levels.

B.2.4 Additional comments

This study discusses the levels of detected chemicals in the blood of named individuals, particularly when these values were higher than others within the survey. This approach is very unusual and raises potential ethical issues relating to the need to maintain patient confidentiality and to provide patient follow up if a significant risk is considered to exist. No mention is made of the consent procedures adopted that would allow such practice to be followed.

B.3 Greenpeace Netherlands

B.3.1 Description of the study

The 2005 biomonitoring survey 'A Present For Life' (Greenpeace Netherlands, 2005) analysed blood samples taken from the umbilical cords of 27 new born babies and 42 new mothers in the Netherlands. The samples were analysed for 35 chemicals (covering 8 chemical groups: alkyl phenols, artificial musks, bisphenol-A, brominated flame retardants, organochlorine pesticides, perfluorinated compounds, phthalates and triclosan). The report contains the conclusions of the study but minimal supporting test results. The study reports finding that "many hazardous chemicals are present in maternal as well as cord blood" and that the "levels of certain phthalates, artificial musks, organochlorine pesticides, triclosan and perfluorinated chemicals are of particular interest and concern".

B.3.2 Assessment of the study

Analytical integrity

The analytical integrity cannot be assessed in any way. No details of the sampling protocols or analytical techniques were reported, although the contracted analytical laboratory would be expected to provide accurate results. Similarly, no information on quality control considerations was reported, including limits of detection.

Ability to describe exposure

The study provides no useful ability to describe exposure: it is not reported how the study subjects were selected. No attempt was reported as having been made to obtain exposure histories that might help explain the nature of the findings. Reporting of exposure data is confined to basic information e.g. number of samples contained within an (arbitrary) identified exposure range (at the ng/g level). The raw data are unavailable.

Ability to relate exposure parameters to effects

The study was not intended to provide any ability to relate the measured exposure parameters to effects. It simply summarises the general findings for the new mother and baby groups and speculates what the source of the exposures might be.

Weight of evidence that observed effects are related to the exposure

The report makes reference to previous Greenpeace and WWF reports and selected other references in order to put the findings in context. However, this attempt at a weight of evidence comparison is only possible to perform when more detailed statistics (and ideally the full data) are available. Moreover, comparison with only a limited series of studies, when many others exist, is biased.

B.3.3 Applicability in risk assessment

The study has almost no role to play in the risk assessment process. Key information on exposure and effects is lacking. A key conclusion of the study that "exposure of the foetus to a continuous low dose of a complex mixture of chemicals is a serious cause for concern" cannot be substantiated.

B.3.4 Additional comments

This study measures the levels of chemicals found in cord blood. The procedures used to obtain and analyse such samples can differ from those for adults. It is therefore surprising that no description of these procedures is contained within the report.

B.4 CDC

B.4.1 Description of the study

The third national report on human exposure to environmental chemicals (CDC, 2005) is probably the largest human biomonitoring study ever reported. The report provides information on 148 chemicals, which were grouped in the following categories: 1. metals, 2. tobacco smoke (cotinine), 3. polycyclic aromatic hydrocarbons (PAHs), 4. polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and co-planar and mono-ortho-substituted polychlorinated biphenyls ('dioxins-related compounds'), 5. non-dioxin-like polychlorinated biphenyls, 6. phthalates, 7. phyto-estrogens, 8a. organochlorine pesticides, 8b. organophosphate pesticides, 8c. carbamate pesticides, 8d. herbicides, 8e. pyrethroids, 8f. other pesticides. In a previous report, biomarker data on 110 of these chemicals were also reported. Chemicals for which data were published for the first time included a number of dioxin-related compounds, a number of PAHs, some phthalates and all pyrethroids. The blood and/or urine samples were obtained in the period 1999-2002. The sample size per chemical analysed varies from about 1200 to 9000 individuals. For analysis the data were subdivided according to gender, age (4 age groups: 1-5 years, 6-11 years, 12-19 years and 20 and older) or ethnicity and compared to data obtained in previous national surveys.

B.4.2 Assessment of the study

Analytical integrity

Extensive lists with references on the analytical methodology used for the assays are provided in the report along with descriptions of the quality assurance, quality control and statistics applied and tables with the limits of detection for the various assays. Although the analytical integrity cannot directly be assessed based on the data provided in the report, it seems that all methodologies are well validated and properly applied.

Ability to describe exposure

It is not possible to relate the measured biomarkers to exposure because of the nature of the study. However, the data are useful to analyse exposure trends for those chemicals for which biomarkers were measured in previous studies using the same analytical methodology.

Ability to relate the measured exposure parameters to effects

Only for a very limited number of chemicals, such as the heavy metals lead and cadmium, for which a good understanding of the health risks associated with different levels of biomarkers exists, can the biomarkers be related to effects. The report itself, however, concludes that "research studies, separate from the report, are required to determine which blood or urine levels are safe and which are associated with disease".

Weight of evidence that observed effects are related to the exposure

Not applicable to these studies since no health effects were observed or reported.

B.4.3 Applicability in risk assessment

The study has a very limited role to play in the risk assessment process since the human database is lacking for almost all of the chemicals for which biomarkers were measured. For a limited number of chemicals, however, animal data are available on dose-effects relationships and toxicokinetics. In these cases a limited risk assessment might be feasible.

B.4.4 Additional comments

The large database on biomarker data of high analytical quality obtained in a well-described population, in combination with the fact that the measurements are repeated over time, provides an excellent opportunity for exposure trend analysis. The results of the studies, indeed, indicate for instance a reduction in the percentage of children aged 1-5 years with elevated blood lead levels from 4.4% in the early 1990s to 1.6% in the period 1999-2002. Exposure to environmental tobacco smoke, as measured by the determination of cotinine in urine of non-smoking individuals, was lower by 68% for children, 69% for adolescents, and about 75% for adults in 1999-2002 compared to 1988-1991. Levels of cotinine in non-Hispanic blacks were more than twice those of Mexican Americans and non-Hispanic whites. Children's levels were more than twice those of adults. Another example is the exposure to organochlorine pesticides such as aldrin, endrin and dieldrin, which were once widely used insecticides in the USA. Agricultural uses of aldrin and dieldrin were discontinued in the USA in 1970, and their use for termite control ended in 1987. Production and use of endrin was discontinued in 1986. Low blood levels of these pesticides were detectable in virtually every individual in the 1980s. The present studies, on samples collected in 2001 and 2002, however, show that aldrin and dieldrin are undetectable in all individuals aged less than 20 and undetectable in most individuals over 20. In fact, aldrin and

dieldrin were only detected in 10% of the population and then only at serum levels lower than recorded in 1999 and 2000.

B.5 Perfluorooctane sulfonate and related fluorochemicals

B.5.1 Description of the study

Perfluorinated alkyl compounds are present in consumer products such as carpets, textiles, paper coatings and surfactants). They occur as mixtures in the environment. They are found in wildlife all over the world (fish and birds). The compounds are persistent and accumulate in liver and blood. In the presented study, 473 blood/serum/plasma samples from the general population recruited in 9 different countries were analysed (Kannan *et al*, 2004). Among the perfluorochemicals measured, perfluorooctane sulfonate (PFOS) was the predominant compound found in blood. Concentrations of PFOS were the highest in the samples collected from the United States and Poland (> 30 ng/ml); moderate in Korea, Belgium, Malaysia, Brazil, Italy, and Colombia (3 to 29 ng/ml); and lowest in India (< 3 ng/ml). Perfluorooctanoate (PFOA) was the next most abundant perfluorochemical in blood samples; concentrations of perfluorohexane sulfonate (PFHxS) and perfluorooctanesulfonamide (PFOSA) were 5 to 10 times lower than PFOS. No age- or gender-related differences in the concentrations of PFOS and PFOA were found in serum samples. The degree of association between chemicals varied depending on the origin of the samples.

B.5.2 Assessment of the study

Analytical integrity

The analytical method used (HPLC MS/MS) and the quality criteria are indicated. No internationally accepted standardised procedure exists for the analysis of these compounds, but the performance characteristics of the method used are given. Blood/serum/plasma (0.5-1 ml) is used as starting material, internal standards are used, precision and accuracy estimates are given, and recoveries are determined. Solvents, materials and recipients are checked for contamination, procedure blanks are included, and LOD and LOQ are determined.

Ability to describe exposure

This is the first study that gives an indication of internal exposure levels of perfluorinated alkyl compounds in humans in different parts of the world. The samples were collected from Red Cross blood centres and university hospitals, but the sampling strategy is not well described. No

stratification schedule was implemented with regard to geographical areas, gender, age group or life-style factors. Hence selection bias cannot be excluded and the study population cannot be considered as representative for the selected geographical areas. Different measures are given to describe internal exposure (mean/median/range/percent positive values above LOQ). Data are stratified by country and by gender. Age effects and gender effects are studied but not evaluated as statistically significant. No information in relation to external exposure is obtained except for the correlation among different fluorochemicals, which varies between donors of different countries. This may indicate a multitude of sources, with varying levels and compositions of perfluorochemicals, and varying exposure patterns in different countries. No information on kinetics and exposure pathways is available.

Ability to relate the measured exposure parameters to effects

The ability to relate the measured exposure parameters to effects is limited. The human database is almost non-existent. It is known from experimental studies that some perfluorinated alkyl compounds such as PFOS have multiple biological targets. Animal experiments indicate potency for liver toxicity, cancer and reproductive toxicity (Seacat *et al*, 2003; Covance Laboratories, 2002; Lau *et al*, 2004).

Weight of evidence that observed effects are related to the exposure

The database of perfluorinated compounds is still limited. The present study does not contribute to the weight of evidence to relate exposure to health effects.

The OECD published a hazard assessment of PFOS and its salts in 2002 (OECD, 2002). In 2003, the United States Environmental Protection Agency (US EPA) released a preliminary risk assessment of the developmental toxicity associated with exposure to perfluorooctanoic acid and its salts (US EPA, 2005). The OECD concluded that PFOS is persistent, bioaccumulative and toxic to mammalian species. The OECD identified a NOAEL of 0.1 mg/kg bw/day, based on the results from a two-generation study in rats.

B.5.3 Applicability in risk assessment

Whilst this study contributes valuable information on exposure levels of the general population, this is only part of the information needed for risk assessment.

B.5.4 Additional comments

In 2000, one of the producers of these compounds has voluntarily decided to phase out the production of perfluorooctane sulfonate and its precursors and to find substitutes for PFOS chemistry.

B.6 Hexachlorobutadiene

B.6.1 Description of the study

The study by Staples *et al* (2003) assessed the prevalence of proteinuria (3 proteins and 5 enzymes linked to renal effects) in a population that used to live in a contaminated industrial area on two occasions (2 months and at least 10 months) after it was moved to another area. No specific exposure measurements were performed in this study but previous investigations had shown that several volatile industrial chemicals were present at the location, including hexachlorobutadiene (HCBD). HCBD was measured in the atmosphere in some homes at levels of up to 6.8 ppb and in a single case 1 ppm was measured. At group level, proteinuria was increased at 2 months compared to the reference value. At about 10 months after the move, proteinuria was significantly decreased compared to the values measured 2 months after the move. It was concluded that there was a renal effect, which improved by moving the population from the contaminated area. This suggested a local environmental factor being responsible for the observations that would be consistent with the predicted toxicological effects of HCBD from animal studies.

B.6.2 Assessment of the study

Analytical integrity

The analytical integrity cannot readily be assessed as only sparse data on the methodology were reported. No quality control or use of reference material was reported, but the methods appear to be applied on a routine basis. Since the investigations were set up as an intervention study, the accuracy (absolute values) of the results is less important, but should be used with caution when compared to data from other studies.

Ability to describe exposure

The ability to describe exposure was relatively weak. The study subjects lived in an area with a legacy of industrial contamination. Two former sandstone quarries in the village of residence of

the study subjects had been used for disposal of industrial wastes. It is very likely that the subjects have been exposed to low levels of a myriad of industrial chemicals since some chemicals (by default limited to those analysed for) were reported to have leached into the groundwater. The study concentrates on HCBD as this chemical apparently came up in a hazard assessment and was found at ppb levels during some indoor air quality investigations.

Ability to relate the measured exposure parameters to effects

The ability to relate the measured exposure parameters to effects was, in principle, limited because only one single chemical was assessed whilst it is more than likely that various chemicals may have been present. In fact, the observed effects may be due to HCBD, to another chemical that happens to be present as well, or perhaps most likely, to a combination of exposures.

Weight of evidence that observed effects are related to the exposure

The weight of evidence indicates that the observed effect is probably due to exposure since the study was designed as an intervention study. However, the exact nature of the exposure in terms of substances is uncertain.

B.6.3 Applicability in risk assessment

The set-up as an intervention study design takes away a lot of the uncertainties often associated with biomarkers of effect. Moreover, this design shows how biomarkers can be applied effectively even if the link between exposure (to a specific substance) and effect cannot be established. The study is inadequate for the risk assessment of a specific chemical despite the link made by the authors to HCBD exposure.

B.6.4 Additional comments

Despite the fact that individual results were measured and it was established which persons were outside the pragmatically defined ‘normal range’, the authors did not mention anything about how individual results were reported back to the persons who took part in the study.

B.7 Fluazifop-butyl

B.7.1 Description of the study

Fluazifop-butyl (FB, trade name Fusilade) is a post-emergence herbicide used to control grass weeds in a large variety of broad-leaved crops. Biological monitoring studies were required to assist in risk assessment processes. A human volunteer study was carried out to measure excretion of the acid metabolite of FB (Woollen *et al*, 1991). This showed that between 80 and 93% of the dose was excreted in urine as fluazifop acid over a period of 6 days. The elimination could be described as a one-compartment model and based on plasma and urine data the elimination half-life ranged from 9 to 37 hours. In a second study (Ramsey *et al*, 1992) three different concentrations (2.5, 25 and 250 μg/cm^2) of FB were administered to an 800 cm^2 area on the back of volunteers. Fractional absorption was dose-dependent, with the total amount absorbed accounting for 8.0, 3.4 and 1.6% of the applied dose of the low, intermediate and high concentrations, respectively. A field application study was carried out in which FB was applied to field crops such as sugar beet and potatoes using tractor-mounted spray equipment (Chester *et al*, 1990). Biomonitoring for fluazifop acid involved 24-hour urine collections from the day before exposure up to 10 days following exposure. The conversion factor for FB equivalent dose was taken from the oral human volunteer studies. The results showed excellent agreement of the absorbed dose with the dermal volunteer studies and also demonstrated the beneficial effects of wearing gloves during mixing and loading. In the group of workers who used gloves, an average exposure of 2.7 mg resulted in an absorption of 6.2%. A second group without gloves incurred an average exposure of 22 mg, of which 3.1% was absorbed.

B.7.2 Assessment of the study

Analytical integrity

The analytical integrity cannot be assessed readily as this method was developed in house and was not subject to external validation. However, it has given consistent results in separate studies that were performed several years apart.

Ability to describe exposure

The ability to describe exposure lies at the heart of this work and can be considered to be high since the results from field biomonitoring studies could be related back to oral and dermal toxicokinetics obtained from human studies.

Ability to relate the measured exposure parameters to effects

No attempt has been made to relate the measured exposure parameters to health effects. However, this can be done in a risk assessment process where exposure as measured in biomonitoring studies is compared to a relevant NOAEL from a laboratory study (see below).

Weight of evidence that observed effects are related to the exposure

This is not applicable for these studies since no health effects were observed.

B.7.3 Applicability in risk assessment

A tiered approach is used for the generation and use of human data for the purpose of pesticide operator risk assessment (OECD, 1997). Each tier involves the comparison of an exposure data set with the appropriate NOAEL and applying an assessment or safety factor to account for uncertainties when extrapolating from animal data to human exposure, as well as inter-individual differences in human response. The first tier in the risk assessment process would typically involve the use of generic databases that allow model calculations for the likely exposure level. At this stage, very conservative assumptions are used (e.g. no protective equipment, 100% skin absorption). In the second tier the data set is refined, for example using actual skin absorption data (animal or human *in vitro*) and building in factors to account for personal protection. The third tier involves the generation of actual human data and biomonitoring backed by human toxicokinetic information and is considered the most relevant method. This study with FB is a good example of how such a process can be supported by good quality human data.

B.7.4 Additional comments

A physiologically-based mathematical model of skin absorption in humans was developed to better understand the factors influencing dermal absorption of chemicals and to be able to predict absorption from a range of given parameters (Auton *et al,* 1994). Part of the FB data set was used to estimate unknown model parameter values; the remainder was used for partial validation of the model. The model takes into account the fact that only a small fraction of the dose was absorbed, since most of it was removed onto clothing and by washing.

B.8 DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane)

B.8.1 Description of the study

From a cohort of 44,000 eligible children born between 1959 and 1966 a subset of 2,380 children was selected for which a mother-child pair could be formed with a complete data set. These mother-child pairs were studied to see whether there was an association between maternal serum concentrations of DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), the main metabolite of DDT, at the time of pregnancy and for any pre-term birth (Longnecker *et al*, 2001). From the 2,380 children selected, 361 were born pre-term and 221 were small for gestational age at time of birth. The median DDE concentration in maternal serum was 25 μg/l, which is one to two orders of magnitude higher than the levels currently found in the general population. The adjusted odds ratios for pre-term births increased with increased serum concentrations and the trend was statistically significant ($p < 0.0001$). The adjusted odds for small-for-gestational-age also increased with maternal serum DDE but the trend was less consistent ($p = 0.04$). The authors conclude that the results strongly suggest that DDT use increases pre-term births, which is a major contributor to perinatal mortality.

B.8.2 Assessment of the study

Analytical integrity

The analytical integrity cannot be readily assessed as the serum samples were stored frozen for 35-40 years in glass at -20°C under poorly documented conditions, e.g. it is stated that the number of thaws was not recorded. However, DDT and DDE have been reported to be extremely stable under a variety of circumstances. The paper refers to another paper for methodology and this paper describes proper quality assurance and control. However, it also states that recoveries according to their method vary from 40-80% whilst the data by Longnecker and co-workers are, as explicitly stated, not corrected for recovery. This means that the actual serum concentration may have been twice as high as reported.

Ability to describe exposure

The ability to describe exposure is limited since only a single sample was analysed for each mother-child pair (with uncertain results as described above). However, a small subset of 67 samples showed a fairly constant serum DDE level when the first and third trimester of the pregnancy was compared. Moreover, this type of chlorinated pesticides has a very long half-life and therefore serum levels are likely to be fairly constant when the main source of exposure is through diet and environment.

Ability to relate the measured exposure parameters to effects

There was a clear relationship (trend) between the maternal serum levels of DDE and the incidence of pre-term births.

Weight of evidence that observed effects are related to the exposure

The authors hypothesise that DDE may hamper the binding of androgen to its receptor and might therefore cause androgen insensitivity. They also suggest that it may affect the binding of progesterone to its receptor, which, in turn, might influence duration of gestation. They sustain this hypothesis with the experimental finding that female mice, bred to have androgen insensitivity, have smaller litters and a reduced reproductive life and the fact that o,p'-DDT (a minor component of DDT which was not measured in the study) has weak oestrogenic properties, whilst the strong oestrogen DES (diethylstilbestrol) is a strong risk factor for pre-term births. All taken together, this seems a very weak hypothesis. In addition, the authors warn for the fact that if high serum albumin would be associated with pre-term birth, the results could be strongly confounded since it is known that DDE is strongly bound to albumin and that high albumin levels therefore would lead to high serum DDE levels.

B.8.3 Applicability in risk assessment

Despite the fact that there are statistically significant trends in the correlation between maternal serum DDE levels during pregnancy and pre-term birth, a causal relationship can only be hypothesised. The underlying animal studies do not provide a solid basis for the postulated theory. Moreover, only a small subset of the eligible children was selected (5.4% of the total cohort) based on the availability of a complete dataset of the mother-child pair. This may have introduced a selection bias. In addition, the investigators also recorded greater frequencies for pre-term births in black children, mothers with a lower socio-economic status and mothers who smoke, which strongly suggests that other confounders may play a major role. It can also not be ruled out that these factors are not-causally related with higher serum DDE levels.

B.8.4 Additional comments

A more recent study (Cohn *et al*, 2003) studied 646 daughters born between 1960 and 1963 from mothers who provided a serum sample at their birth to investigate the association between serum DDT and its metabolites in the mothers at time of birth and fecundity in the daughters. In total 289 women were eligible and showed an increased fecundity with increasing levels of maternal DDE. The authors speculate that the anti-androgenic activity of DDE mitigates the harmful

effects of DDT and explains the absence of dramatic changes in human reproductive performance after the introduction of DDT. The studies by Cohn *et al* and Longnecker *et al*, using the same biomarkers and similar set up, and using similar biological explanations loosely to explain their results, show clearly that biomarker studies may be very useful to generate hypotheses. However, these need scrutiny and additional investigative studies with respect to the biological mechanisms before hints of causal relationships are suggested.

B.9 Vinclozolin

B.9.1 Description of the study

The cross sectional study of 67 men engaged in pesticide synthesis and formulation operations and 52 controls by Zober *et al* (1995) was designed to examine internal exposure and targeted health outcomes of employees exposed to vinclozolin, a fungicide used to protect a variety of agricultural and horticultural products. Biomonitoring was based on determination of urinary metabolites that contained a 3,5-dichloroaniline moiety. Targeted health endpoints were the same as in previous animal studies - namely, reversible changes in the concentrations of various hormones, signs of liver injury, haemolytic anaemia, cataract formation and hormonally induced hyperplasia and tumours. The clinical investigation consisted of a medical and occupational history questionnaire, physical examination, laboratory determinations (including testosterone, luteinising hormone, and follicle stimulating hormone measurements) ultrasonication of samples of liver tissue and prostate tissue, a detailed eye examination, and routine spirometry. The biomonitoring results revealed, that two thirds of the study group exceeded an equivalent of the vinclozolin acceptable daily intake (ADI) used for consumer regulatory purposes, which was clearly kept by the (non-occupational exposed) control group. Even the highest concentrations were, however, far below the NOAEL based on animal studies. Analysis of physical examination and laboratory data provided no evidence of hormonal responses induced by vinclozolin. Furthermore no evidence of liver injury, prostate changes, cataract formation, or haemolytic anaemia was found.

B.9.2 Assessment of the study

Analytical integrity

The analytical integrity cannot be assessed directly as only sparse data on the methodology were reported in the paper (as is usual in medical journals). The analytical method was published elsewhere and quoted.

Ability to describe exposure

The ability to describe exposure is verified by:

- animal experiments quoted in the publication of the analytical method;
- the results published in the paper;
- the findings about excretion kinetics published in the paper.

Ability to relate the measured exposure parameters to effects

The ability to relate the measured exposure parameters to effects is limited due to the overall outcome of the study, i.e. that there was no evidence of any health effects induced by vinclozolin. But it is possible to relate urinary excretions of metabolites to the dose of vinclozolin and thus, indirectly to effects in animal studies.

Weight of evidence that observed effects are related to the exposure

The weight of evidence indicates that in spite of a distinct exposure to the chemical of concern, no health effects occurred.

B.9.3 Applicability in risk assessment

Based on the quantitatively known metabolic pathway of vinclozolin, biomonitoring results can be correlated to the internal dose and thus, to targeted health endpoints in previous animal studies. Thus, the chosen biomarker can be applied effectively even if the link between exposure and effect cannot be established in the human study but only in animal studies.

B.9.4 Additional comments

Because of the detailed analysis of the working conditions and the lowering of the ADI in 1995 and 2000, continuous improvements were made in the process of manufacture and formulation as well as in the protective equipments. The success of these measures became evident in several follow-up investigations (Will and Hoffmann, 1996; Will, 2005a,b).

B.10 Methyl eugenol

B.10.1 Description of the study

The studies of Barr *et al* (2000) and Schecter *et al* (2004) assessed serum methyl eugenol biomarkers in a subset of the US population in the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994). The US National Toxicology Program evaluated methyl eugenol administered by the oral gavage route in a two-year bioassay (NTP, 2000). The NTP concluded, based on the bioassay results, that there was clear evidence of carcinogenicity at every dose level tested, based on liver tumours in rats and mice. Although toxicological endpoints have been established in animals, no similar effects have been associated with consumption of methyl eugenol in humans. This particular case is of interest since it offers an opportunity to evaluate a naturally occurring substance in the context of biomonitoring. Currently, there is no known association between methyl eugenol exposure and adverse health effects in humans.

B.10.2 Assessment of the study

Analytical integrity

Several studies have evaluated biomarkers of methyl eugenol in animals, primarily rats, however only one method has been reported to measure this substance in humans (Barr *et al*, 2000). This method uses serum as a source to measure intact methyl eugenol. Isotopically labelled methyl eugenol was used as an internal standard. The methyl eugenol was isolated from the serum using a general solid phase extraction and was measured in the extract using gas chromatography-high resolution mass spectrometry. Although the method was highly selective and sensitive, a residual contamination in the laboratory air and water made the analysis difficult. The method had appropriate sensitivity to measure methyl eugenol in serum, although the levels were quite low. An added benefit of measuring methyl eugenol in blood was the specificity of the marker for determining exposure to methyl eugenol itself. The measurement of metabolites can often be less selective because common metabolites may be derived from structurally similar substances. Although urinary 1-hydroxy methyl eugenol and its glucuronide-bound analogue are potential biomarkers for human exposure to methyl eugenol, there are no published methods for their measurement (Smith *et al*, 2002).

Ability to describe exposure

Limited information exists on specific sources of exposure to methyl eugenol. Since it is a component of many herbs and spices, differences in dietary habits along with the natural variation in levels of methyl eugenol in plants (Smith *et al*, 2002; Di Cesare *et al*, 2003) makes a definitive

prediction of exposure difficult. Based on known levels in foods, spices and herbs the Flavour Extract Manufacturer's Association (FEMA) estimated that methyl eugenol consumption from food is about 5-6 µg/kg bw/day (Smith *et al*, 2002). In addition, use of essential oils as flavouring agents has resulted in a total methyl eugenol exposure of no more than about 10 µg/kg/day. However, ethnic or cultural dietary habits may result in substantially higher exposures to methyl eugenol; one estimate indicates as much as 250 µg methyl eugenol/kg/meal from a pesto meal (Miele *et al*, 2001). The biomonitoring data indicate that serum levels resulting from integrated exposure to methyl eugenol may be as high as 390 pg/g serum (Barr *et al*, 2000; Schecter *et al*, 2004). Using standard human physiological assumptions for blood serum levels, this translates to an exposure of about 4-12 µg/kg bw/day, which is similar to the estimate of 5-6 µg/kg bw/day presented by Smith *et al* (2002).

Ability to relate the measured exposure parameters to effects

There are accurate determinations of methyl eugenol in serum, but there are no human data indicating that chronic exposure to dietary levels of methyl eugenol has any adverse health effect.

Weight of evidence that observed effects are related to the exposure

No link has been established between methyl eugenol consumption and health effects in humans. However, it is worthwhile to compare human exposure based on the biomonitoring data to the available rodent bioassay data. A recent study provides some toxicokinetic information following methyl eugenol ingestion by humans (Schecter *et al*, 2004). Volunteers consumed ginger snap cookies containing approximately 18 µg methyl eugenol/cookie, with total consumption of about 216 µg. The level of methyl eugenol in the blood peaked at about 15 minutes and the half-life was estimated to be about 2 hours (Schecter *et al*, 2004). The exposure from the Schecter *et al* (2004) study translates to about 4 µg/kg, a value very comparable to the prior estimates of methyl eugenol exposure of 5-6 µg/kg (Smith *et al*, 2002) and to the 3.1 µg/kg derived from biomonitoring data (Barr *et al*, 2000).

B.10.3 Applicability in risk assessment

There are hazard identification data indicating that methyl eugenol induces hepatocellular carcinomas in rats and mice. However, the question remains as to the relevance of the results since the animals received substantial bolus doses that are not representative of typical methyl eugenol exposure. In addition, the higher doses caused significant mortality supporting the hypothesis that the high doses may have overwhelmed the primary detoxification capabilities of the animals (Smith *et al*, 2000). This raises the likelihood that significant levels of a 1'-hydroxyl

reactive metabolite were present. In contrast, at typical dietary exposures this 1'-metabolite would either not be formed or be formed in extremely small amounts. Importantly, there is no association between human exposure to methyl eugenol and disease. A detailed literature review has indicated that no human epidemiology studies have been conducted to evaluate the association of diets containing a high level of methyl eugenol and any type of health outcome.

B.10.4 Additional comments

There are other potential sources of exposure to methyl eugenol including agriculture (Vargas *et al*, 2000), consumption of wine (De Simon *et al*, 2003) and as part of the ambient background in air and water (Barr *et al*, 2000). There have been no analyses of occupational or lifestyle impact on serum levels of methyl eugenol.

B.11 Use of biomonitoring for assessing environmental exposures and effects

B.11.1 Description of the study

This study evaluated the extent to which biomarkers can reliably measure exposure to, and health effects of, common environmental pollutants (Staessen *et al*, 2001). 200 adolescents were recruited from a rural area and from two suburban areas. Biomarkers of exposure and effects were measured in blood and urine. Participants filled in questionnaires with information on lifestyle and socio-economic factors, on health and dietary habits. Clinical investigations included morphometry and sexual maturation stage.

B.11.2 Assessment of the study

Analytical integrity

The analytical laboratories met national and international quality standards for most biomarkers of exposure that were measured (Pb and Cd in blood, Cd in urine, urinary *trans,trans'*-muconic acid and orthocresol as metabolites of benzene and toluene respectively, 1-hydroxypyrene in urine and marker PCBs in serum). Dioxin activity was measured by the CALUX bioassay, which is not yet a fully validated method but the method has been published (Koppen *et al*, 2001). Biomarkers of effect, such as cystatin C in serum (glomerular kidney function), β-microglobuline in urine (tubular kidney function), chromatid breaks (DNA damage) and urinary creatinine, were measured according to internationally accepted and fully validated and methods. Some newer methods (alkaline comet assay and urinary 8-hydroxydeoxyguanosine) were in-house validated

methods, and the protocols have been published. Clinical measurements were performed by four school doctors and may have been subject to inter-observer variability.

Ability to describe exposure

Biomarkers were expressed as geometric means with 95% confidence limits. They were adjusted for confounders (sex, BMI, smoking etc.). Data were stratified by gender and recruitment area. Information on pollution pressure in each of the study areas was limited. Information on kinetics and exposure pathways was not given in the manuscript. Without doubt the participants in the study were subject to multiple chemical exposure, the selected biomarkers have to be considered as markers for selected environmental stressors (traffic exposure, industrial emissions etc).

Ability to relate the measured exposure parameters to effects

Single and multiple regression models were used to calculate dose-effect relationships according to hypotheses that were put forward in advance and taking into account confounders and covariates. Levels of lead in blood were significantly associated with renal effects. 1-hydroxypyrene in urine was significantly associated with results of the comet assay, chromatid breaks and chromosome aberrations. Orthocresol in urine was associated with 8-hydroxy-deoxyguanosine in urine and with the comet assay results. PCBs and dioxin like compounds in serum were associated with sexual maturation stage. Effect size was calculated. However one must keep in mind that this study describes associations between biomarkers of exposure and biomarkers of effect but no proof of causality is given by this study. The effects may also be attributable to many causes, not all of which are controlled. Only a few chemicals are measured in this study. Although it is known that environmental exposure is always to mixtures, the observed effects may not be specific for the chemicals under study.

Weight of evidence that observed effects are related to the exposure

The information obtained in this study should be considered together with information from other epidemiological studies and from animal studies (see also Den Hond *et al*, 2002; Van Den Heuvel *et al*, 2002). As such, this study contributes to the database needed for risk assessment.

B.11.3 Applicability in risk assessment

The authors concluded that biomarkers can be used to detect environmental exposure to pollutants and measure biological effects before overt disease develops. The authors also suggest

that current environmental standards are insufficient to avoid measurable biological effects. The remarks made above, concerning the uncertainty to be able to relate the effects to exposure, should be kept in mind. Also, the extent that the observed biological effects are related to adverse health outcomes requires further investigation.

B.11.4 Additional comments

As a follow up to this pilot study, the Flemish authorities responsible for environment and health have initiated a programme to install a human biomonitoring network in order to evaluate environmental health pressure in different geographical areas. The network is used as a surveillance instrument to follow up environmental health threats and to evaluate the efficacy of environmental health measures.

B.12 Aflatoxin-N-7-guanine (AFB-NZ-G)

B.12.1 Description of the study

The studies of Ross *et al* (1992) and Qian *et al* (1994) assess urinary aflatoxin B1 biomarkers in a population of 18,244 men in Shanghai, People's Republic of China. After nearly 70,000 person years of follow-up following the urine collection, there were 55 cases of hepatocellular carcinoma (HCC). Urinary aflatoxin B1 metabolites and its major nucleic acid adduct, aflatoxin-N-7-guanine (AFB-N7-G) were measured by HPLC after immunoaffinity separation of the analytes. Highly significant correlations were found between the presence of urinary aflatoxins, or of serum hepatitis B surface antigen (HBsAG) positivity, with liver cancer risk. Of particular interest was the high association of the adduct concentration with disease outcome. There was a strong interaction between the risk factors of urinary aflatoxin biomarkers and HBsAG, the combined relative risk being 59.4 for HCC. There was no significant association between HCC and estimated dietary aflatoxin intake.

B.12.2 Assessment of the study

Analytical integrity

The study was based on sophisticated analytical techniques and the use of immunoaffinity purification indicates that the approach has high selectivity. Authentic standards were available to establish calibration curves. The method was developed ‘in-house’ and there are no international validation procedures for it.

Ability to describe exposure

Groopman *et al* (1992a) showed that there is a direct proportionality between dose of aflatoxin B1 given to rats and excretion of AFB-N7-G. Urinary AFB-N7-G is also related to dietary intake of aflatoxin B1 in human subjects (Groopman *et al*, 1992b). These data support the use of the measurement of AFB-N7-G as a measure of dietary aflatoxin exposure in this study. Exposure from other sources was not expected to be significant.

Ability to relate the measured exposure parameters to effects

In rats AFB-N7-G excretion reflects DNA adduct levels in rat liver (Groopman *et al*, 1992a). As liver aflatoxin B1-DNA adduct levels correlate with tumour incidence in animals there is strong experimental indication for the use of urinary AFB-N7-G as a biomarker of effect. This is supported by this nested case-control study showing a relationship between AFB-N7-G and HCC incidence.

Weight of evidence that observed effects are related to the exposure

There is statistical evidence in these studies that the presence of the aflatoxin adduct in urine is a predictor of the adverse health effect, HCC. However HBsAG status is also a risk factor. This appears to interact strongly with the urinary aflatoxin biomarker.

B.12.3 Applicability in risk assessment

It is known from epidemiological studies that there is a relationship between aflatoxin ingestion and HCC. The known correlation of urinary AFB-N7-G with exposure and with biological effects supports the use of AFB-N7-G in the risk assessment process, when considered in conjunction with HBsAG status.

B.12.4 Additional comments

Some chemo-preventive agents are known for aflatoxin carcinogenicity. Thus, for example, chlorophyllin reduced aflatoxin B1-DNA adducts in rats and it protected against hepatocarcinogenesis in animal models. The urinary excretion of the aflatoxin adduct biomarker has been investigated in human populations treated with this chemo-preventive agent and it has been demonstrated that significant reductions of the excreted adduct are achievable after

treatment. It is hoped (on the basis of the conclusions from animal experiments) that these reductions in humans will be associated with a decrease in tumour incidence (Egner *et al*, 2001).

B.13 Chlorinated hydrocarbons

B.13.1 Description of the study

The study by Boogaard *et al* (1993) is a biological effect monitoring study on operators of a petrochemical plant producing chlorinated hydrocarbons. The aim of the study was to investigate whether prolonged exposure to low levels of chlorinated hydrocarbons might lead to early, sub-clinical effects in hepatic and renal function. The study group comprised 73 workers that had been exposed to a number of chlorinated hydrocarbons for an average of 8.2 years (range 0.5-23 years) and the control group comprised 35 men employed at the materials division of the same company without occupational exposure to chlorinated hydrocarbons. Biochemical alterations of liver function were assessed by determination in serum of alanine and aspartate aminotransferases, alkaline phosphatase, total bilirubin, γ-glutamyltranspeptidase, lactate dehydrogenase, and total bile acids (SBA). No differences between the exposed group and the control group were found. Nor were differences found in biochemical tests for renal tubular damage (urinary alanine aminopeptidase and N-acetyl-β-D-glucosaminidase) or renal tubular function (urinary retinol binding protein (RBP). Total urinary protein and albumin excretion were measured to assess the integrity of the glomerulus. Urinary total protein did not differ between the groups, but urinary albumin, although within normal limits in both groups, was significantly higher ($p < 0.02$) in the exposed group. This difference in urinary albumin could not simply be explained by exposure to chlorinated hydrocarbons because albumin concentrations did not correlate with the duration of employment.

B.13.2 Assessment of the study

Analytical integrity

The analytical integrity was assured. All parameters were assessed on a routine basis and all were subject to internal quality control and, with exception of SBA (in experimental phase at the time of the study) and RBP, which was performed elsewhere, but subject to quality control.

Ability to describe exposure

The ability to describe exposure was relatively good for studies of this kind since an exposure database for the preceding years was available for all chlorinated hydrocarbons produced at the

plant (allyl chloride, 1,3-dichloropropene, epichlorohydrin and hexachlorocyclopentadiene). The concentrations of these chlorinated hydrocarbons had been determined regularly by personal air monitoring since 1980. Whilst exposures to 1,3-dichloropropene and epichlorohydrin were well below currently accepted maximum allowable concentrations, relatively high exposures to allyl chloride and hexachlorocyclopentadiene, occasionally exceeding current occupational exposure limits, had been measured in the past.

Ability to relate the measured exposure parameters to effects

The ability to relate the measured exposure parameters to effects had the normal limitations of this type of study: all measured parameters are non-specific so causal relationships can only be assumed but not proven.

The statistically significant effect (i.e. increased microalbuminuria in the exposed workers compared to controls) was graphically clear (frequency curve clearly shifted for exposed compared to controls) but unexpected.

Weight of evidence that observed effects are related to the exposure

The weight of evidence seemed to indicate that the observed effect is not due to the exposure, despite the fact that the historical exposures had been higher than the occupational exposure limits (OEL) at the time of the study, as there was no correlation with duration of employment and because other biomarkers of renal effects were not elevated.

B.13.3 Applicability in risk assessment

Despite the weight of evidence indicating that the observed effect is not due to the exposure (see next paragraph) it is difficult to dispute the assumption that there is a causal relationship, especially if OELs may have been exceeded. In another study by Viau *et al* (1987) a very similar effect, i.e. a marginal but statistically significant increase in microalbuminuria was observed in refinery workers. These authors, however, attributed this effect to hydrocarbon-induced nephropathy.

The resemblance between the two studies is striking both with regard to the frequency curves for microalbuminuria and the complete absence of any signs of other renal effects in the other biomarkers. Follow-up studies in the chlorinated hydrocarbon workers, however, clearly indicated that the most likely reason for the elevated values of the biomarker of effect in the study with operators with potential exposure to chlorinated hydrocarbons is the fact that the ‘exposed’

group worked in shifts whilst the controls were daytime workers (Boogaard and Caubo, 1994). In the study with workers potentially exposed to hydrocarbons the controls were office workers whilst at least half of the 'exposed' group were either shift workers or workers with physically strenuous work (Viau *et al*, 1987). The results of the studies in workers exposed to chlorinated hydrocarbons strongly suggest that the observed effects in the hydrocarbon-exposed workers are due to shift work or physical workload rather than exposure to hydrocarbons.

B.13.4 Additional comments

Since it is almost invariably impossible to relate a certain biomarker of effect to a specific exposure, utmost care must be applied when using them in risk assessment. This is the case even if the observed changes in biomarkers match a harm-hazard relation known from, for instance, animal studies at high(er) exposure.

B.14 Lead

B.14.1 Description of the study

Recently, two studies on risk assessment and risk management based on the concentration of lead in blood samples collected from children were published (Louekari *et al*, 2004; Jarosińska *et al*, 2004). Louekari and co-workers investigated a small number (n = 10) of young children (0-6 years) selected from near the site of a lead smelter with high concentrations of lead in the soil (average 242, range 160-434 mg/kg soil), a somewhat larger group (n = 42) from a moderately contaminated site (average 40, range 15-81 mg/kg soil), and a control group (n = 11) from a reference area (average ~ 20 mg/kg soil). Blood lead levels were compared with regard to the concentration of lead in soil. In addition, a range of other factors were studied, such as lead levels in locally grown foods. Jarosińska and co-workers studied blood lead levels that were measured between 1993 and 1998 in a very large group of children (n = 11,877), aged 24-84 months. These children were selected from 14,000 children from 6 different cities based on the availability of full records (i.e. blood lead levels and questionnaire results). The blood lead concentrations were correlated with a number of variables, including environmental lead levels. The aims of the study by Louekari and co-workers were to define whether children living in a lead contaminated area were actually at risk and to investigate whether soil remediation had been effective. The primary aims of the study by Jarosińska and co-workers were to investigate the sources of lead exposure and the effectiveness of risk reduction measures.

B.14.2 Assessment of the study

Analytical integrity

The analytical integrity was assured as the laboratories used well-established methods for the determination of lead in blood and took part in internal as well as external quality control schemes.

Ability to describe exposure

The ability to describe exposure was good in both studies since internal exposure data were assessed in combination with a variety of external exposure data (ambient air, soil, food). However, the study by Louekari *et al* is limited due to the small number of children investigated, which may have introduced selection bias in the population studied.

Ability to relate measured exposure to effects

It was possible to relate the measured exposure parameters to effects since extensive data are available on blood lead levels in children and adverse health effects whilst all measured values were within the range of published dose-effect relationships.

Weight of evidence that observed effects are related to the exposure

The weight of evidence was not relevant because no adverse health effects were measured and all data (Louekari *et al*, 2004) or the great majority of data (Jarosińska *et al*, 2004) were below the NOAEL for lead in blood.

B.14.3 Applicability in risk assessment

Both studies show the suitability of lead biomonitoring in children in assessing risk and the efficacy of risk reduction measures. In the study by Louekari and co-workers the current lead in blood levels were compared with historical levels from 20 years ago when the lead smelter was closed. Lead content in household dust was still clearly elevated in the contaminated areas and, consequently, blood lead levels of the children living in these areas were slightly, but statistically significantly, higher than those of the children in the control areas. However, the critical blood lead level of 100 μg/l was not exceeded in any of the children examined. Overall, average blood lead levels had decreased by a factor of 3 over the past 20 years, which was described as the

result of a series of specific risk-reduction measures. In the study by Jarosińska and co-workers, airborne lead concentrations and lead fallout, as measured in the ambient air monitoring system, were below current Polish air quality standards. Mean blood lead levels in the children had decreased over time but still more than 13% of children had elevated blood lead levels (> 100 μg/l), with the highest concentration being 480 μg/l, indicating a risk for these children. A number of potential risk factors could be identified to explain this observation such as poor housing, other socio-economic factors and the time spent outdoors.

B.14.4 Additional comments

The study by Louekari *et al* (2004) showed that, despite the fact that the Finnish limit value for lead in soil of 300 mg/kg was exceeded, the blood lead levels of the children living in the contaminated area is below the accepted maximum level. The biomonitoring data reported by Jarosińska *et al* (2004) gave clear indications for potential further risk reduction measures.

ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable daily intake
AFB-N7-G	Aflatoxin-N7-guanine
CDC	US Centers for Disease Control
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DFG	Deutsche Forschungsgemeinschaft
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority
FB	Fluazifop-butyl
FDA	US Food and Drugs Administration
HBsAG	Serum hepatitis B surface antigen
HCBD	Hexachlorobutadiene
HCC	Hepatocellular carcinoma
HESI	Health and Environmental Sciences Institute
HSE	Health and Safety Executive
IPCS	World Health Organisation's International Programme on Chemical Safety
ILSI	International Life Sciences Institute
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
PCB	Polychlorinated biphenyl
RBP	Retinol binding protein
SAR	Structure activity relationship
SBA	Serum bile acid
SCCP	EU Scientific Committee on Consumer Products

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