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IEH report on

THE USE OF BIOMARKERS IN ENVIRONMENTAL EXPOSURE ASSESSMENT

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Institute for Environment and Health
University of Leicester
94 Regent Road
Leicester LE1 7DD

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Executive summary

The investigation of the human health effects of chemical pollutants in the environment, whether exposure occurs through air, water or diet, is currently of considerable interest. Health effects may be small and difficult to distinguish from the normal background of disease, but they may affect a large proportion of the population. Epidemiological investigations and standard setting depend on a knowledge of the level of current and past exposures, both for individuals and populations. It is often easier to establish the disease burden in a population than it is to obtain detailed exposure information; the lack of exposure data may be the major obstacle in establishing a causal relationship between a pollutant and disease.

Exposure to a chemical pollutant may be reflected by an increased amount of that pollutant or its breakdown products (metabolites) in the body fluids or tissues of exposed individuals, or by measurable characteristic biological changes. Increased levels of a pollutant or its metabolites in body fluids may provide a useful index of an individual's exposure for epidemiological purposes. Pollutants, their metabolites or the associated characteristic biological changes are known as *biomarkers* when used in this way. Biomarkers have played a central role in establishing the health effects of environmental exposure to lead, mercury and cadmium. Recent studies of pollution involving complex organic substances, such as the polycyclic aromatic hydrocarbons and dioxins from industrial sites, have been aided by the use of the relevant biomarkers. A number of biomarkers have been developed for monitoring occupational exposure and, as analytical techniques have become more sensitive, they have been used to assess exposure through air outside the workplace as well as exposure through food and water.

Detailed exposure information would help establish the key pollutant, the health effects involved and the effectiveness of any proposed control procedures relating to a number of environmental health issues. Investigation of urban air pollution has depended on ambient monitoring data from a limited number of monitoring sites. The use of biomarkers for individual gases and the particulate fraction of atmospheric samples would allow detailed personal exposure data to be collected and compared with measurements of clinical parameters. These biomarkers are not currently available and need to be developed. Similarly, biomarkers for

combustion gases, nitrogen dioxide and volatile organic compounds would allow comparison of indoor and ambient exposures on an individual basis.

Although biomarkers can potentially offer more detailed information than traditional methods of measuring exposure, there are a number of issues to be considered. While biomarkers may give the best possible measure of external exposure on an individual basis they more accurately reflect an individual's internal dose. An individual's genetic ability to metabolise foreign substances and his/her life-style, diet and medication will all influence the relationship between personal exposure and internal dose (and thus risk). These influences must be taken into account when developing a biomarker approach towards exposure assessment. The relationship between the time of exposure and the measurement of the biomarker is also of critical concern. Some highly volatile pollutants are washed out of the body within minutes of the end of exposure, while cadmium and some of the polychlorinated aromatic compounds can, for example, remain in the body for decades. The usefulness of a biomarker in determining recent or past exposures can only be assessed using a knowledge of the time course of its retention in the body.

In summary, the development of new biomarkers to measure long-term exposure to pollutants should be encouraged, not only for known or suspected carcinogens, but also for neuro- and developmental toxins and hormonally active substances. Furthermore, currently available biomarkers should be used more widely to assess the sources of exposure influencing personal uptake of pollutants.

1 Introduction

This report examines the use of biomarkers as measures of human exposure to chemical pollutants in the environment. It identifies various potential applications, reviews the relevant biomarkers that are currently available or might become available in the foreseeable future, and assesses how far these biomarkers could meet the identified needs.

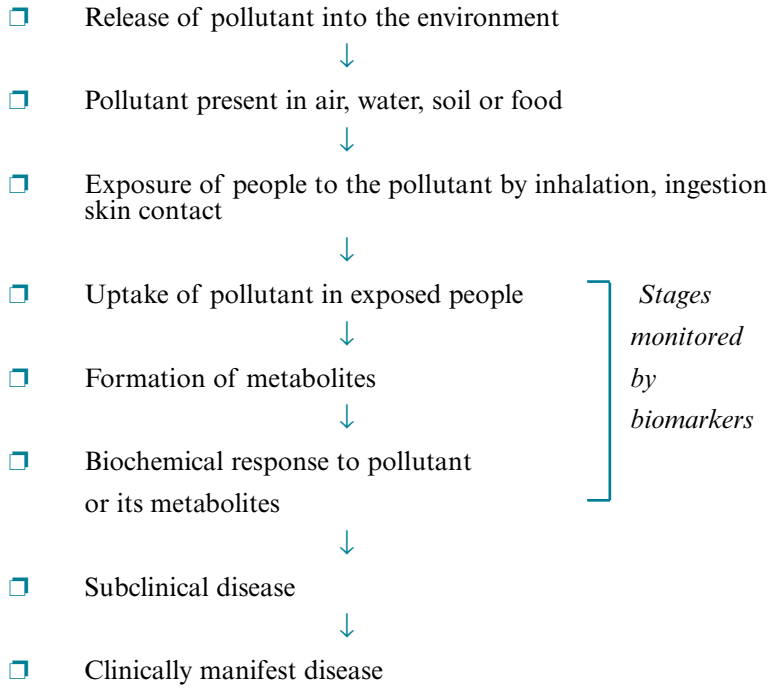
To help in formulating the content of this report, an expert workshop was held in Leicester from 30th November to 1st December 1995 chaired by Dr David Coggon. The names of those who attended are listed at the end of the report. The report includes several papers that were presented at the meeting, and its content is strongly influenced by the discussion that took place. However, it does not necessarily represent a consensus view of the participants.

DEFINITIONS AND SCOPE

For the purposes of this report, a biomarker is defined as a pollutant, a metabolite of a pollutant, or a biochemical response to a pollutant that can be measured in human or animal tissues. Only chemical pollutants have been considered, and only those pollutants that occur in the general environment rather than solely in the workplace.

The process whereby chemical pollutants produce disease can be divided into a series of stages, each of which can be monitored (Figure 1.1). Within this chain, biomarkers are intermediate between exposure and the clinical manifestation of disease. They have potential value as proxy measures of disease outcome (especially for diseases that only follow exposure after a long latent interval), and as a means of distinguishing individuals who may be unusually susceptible to the effects of a pollutant (e.g. because of genetically determined differences in the way that they metabolise the substance). However, this report concentrates primarily on the use of biomarkers as indices of exposure.

Figure 1.1 Stages in the process by which chemical pollutants produce disease



THE NEED FOR MEASURES OF EXPOSURE TO POLLUTANTS

There are several reasons for measuring exposure to chemical pollutants:

- for epidemiological studies to assess the risk of disease in relation to exposure
- to establish the distribution of personal exposures in a population with a view to possible controls on a pollutant

- for studies to assess which sources of exposure determine individual uptake or dose of pollutants (e.g. to assess the relative influences of lead in air and in drinking-water on tissue levels); such information can be extremely valuable in directing control strategies
- to assess the response in a population to controls on a pollutant
- to validate other methods of exposure assessment (e.g. the use of questionnaires).

The requirements for a measure of exposure will vary according to its purpose. For example, in epidemiological studies that use the case–control method to look at long-term effects of carcinogens, the need is for a retrospective index of exposure over many years. In contrast, an investigation of factors determining individual uptake of pollutants will often be better served by a measure that reflects only recent exposures. A study to establish the distribution of personal exposures in a population may need measurements in large numbers of people and the use, therefore, of a technique that is necessarily relatively inexpensive and non-invasive, whereas such limitations might not apply to the same extent in a smaller case–control study.

The accuracy and precision that are required of exposure measurements will also differ according to the application. Where the aim is to assess the distribution of exposures in a population, a degree of imprecision in individual measurements may be tolerable; it would be less acceptable in the investigation of how risk relates to exposure.

BIOMARKERS IN COMPARISON WITH OTHER MARKERS OF EXPOSURE

The role of biomarkers as indices of exposure will depend upon how they perform in comparison with other techniques (e.g. the use of personal air monitoring or questionnaires) for the specific purpose required. New biomarkers may, however, open up novel opportunities that did not exist before. When compared with existing methods, biomarkers offer three advantages:

- They can integrate exposures across all routes. This is the basis for their role in the assessment of how different sources of exposure contribute to the total dose an individual receives. It also means that they can be used to investigate dermal exposure, which is particularly difficult to quantify by other methods.

- They often integrate exposures over longer time periods than can be conveniently studied by other methods. For example, assay of DNA adducts formed by polycyclic aromatic hydrocarbons (PAHs) can provide an index of exposure over weeks or months that would be difficult to achieve with personal air monitoring. In the case of fat-soluble organochlorine compounds, blood levels may reflect exposures over many years.

- Unlike methods based on questionnaires, they do not rely on people's memory.

Against these strengths, however, must be weighed several weaknesses, some of which biomarkers share with existing techniques:

- They are only indirect markers of exposure, reflecting uptake into the body (internal dose) rather than environmental exposure *per se*. They are also influenced by other variables such as individual metabolism and activities (for example, people carrying out heavy physical work have a higher respiratory rate, and therefore tend to take up air pollutants more rapidly when exposed to a given concentration in air). The importance of these other influences will depend on the pollutant, the choice of biomarker and the reason for measuring exposure. Individual differences in uptake and metabolism, however, allow the biomarker to give a more appropriate estimate of individual risk, reflecting the dose actually delivered to target tissues and organs.

- Measurement of some biomarkers is expensive and can only be carried out on a small scale. As well as reducing the range of potential applications, the limitation in the number of samples that can conveniently be analysed restricts the scope for quality control and makes it more difficult to generalise results from one study to another.

- Some biomarkers require invasive procedures such as the sampling of blood, fat or other tissues, and this may restrict their use.

Despite these limitations, many biomarkers have already found an established role in exposure assessment. This is especially so in the workplace, and it is likely that their role in monitoring the non-working environment will extend as technology continues to advance.

THE REPORT

The body of the report that follows is divided into three main sections. Section 2 contains six short papers on special topics related to the use of biomarkers. Section 3 provides an overview of the application of biomarkers in the assessment of exposure to pollutants by different routes and is divided into two parts.

Section 3.1 contains a review of specific issues relating to pollutants in air, water and food. The review covers not only human biomarkers but also biomarkers in animals that might provide information about human exposure. Human exposure to toxic chemicals can be strongly influenced by ecological processes that modulate their distribution. Moreover because of their sensitivity or their link to human food chains, organisms in ecosystems can sometimes provide early warning of hazardous exposure.

In Section 3.2 the most important chemical pollutants in the environment are reviewed. For each substance the main indications for exposure assessment are identified, existing or potential biomarkers are described, and the extent to which these biomarkers could meet the needs for exposure assessment is discussed.

Finally, recommendations and priorities for future research and development are set out in Section 4.

2 Updates on the use of biomarkers in exposure assessment

2.1 BIOLOGICAL MONITORING OF TRACE ELEMENTS TO ASSESS ENVIRONMENTAL EXPOSURE

HT Delves

SAT Trace Element Unit, Southampton General Hospital

2.1.1 INTRODUCTION

Biological monitoring is a well established technique for assessing increased intakes and uptakes of toxic elements following either occupational or environmental exposure. The principal objective is to detect increased concentrations of toxic elements, usually in whole blood or blood plasma or urine, above those found in healthy, unexposed populations, before adverse effects occur. Requirements to achieve this objective include: sensitive analytical techniques; quality control of analytical accuracy and of temporal changes in bias; and an understanding of the physiological and pharmacological responses to increased uptake of the elements in question in order to select the most appropriate specimen.

Recent analytical developments in atomic absorption spectrometry with electrothermal atomisation (ETA-AAS) and of inductively coupled plasma-mass spectrometry (ICP-MS) allow accurate measurements of all stable elements with very small (microlitre) sample volumes. The ability to measure stable isotopes

using ICP-MS is of value in the identification and apportionment of sources of environmental lead exposure. Examples of the use of both of these techniques to assess environmental exposure to toxic metals include: manganese pollution of soils and foods; mercury pollution, from gold-mining activities, of air, water and food; and contamination with lead of soils, dusts and drinking-water.

2.1.2 MANGANESE

This nutritionally essential trace element is an integral component of many enzymes, including pyruvate carboxylase and galactosyl transferase. Manganese deficiency is associated with abnormalities of mucopolysaccharide synthesis and with defective formation of connective tissues, whereas manganese toxicity is associated with neurological disorders involving degeneration of the basal ganglia with predominant gliosis of the *globus pallidus* and parkinsonism. Excessive industrial exposure *via* inhalation of oxide dusts can lead to toxicity states which are manifest by parkinsonian tremors.

Recently, manganese toxicity associated with neurological disturbances has been associated with excessive oral intakes from foods grown on contaminated soils (Cawte & Florence, 1987). Contamination of soil from a surface manganese-ore deposit in Groote Eylandt, Northern Territory, Australia increased the concentrations of manganese in locally grown fruit and vegetables by up to 700 times. The local aboriginal population which consumed these foods had considerably elevated concentrations of manganese in their blood (median 450 nmol/1, range 175–900 nmol/l) compared with a reference population of non-exposed city dwellers (mean 215 nmol/1, range 85–350 nmol/1). A subset of about 2% of the aboriginal population with blood manganese levels of more than 500 nmol/1 had neurological disorders.

The use of manganese determinations was recently questioned by Halls (1994), who particularly queried the choice of a reliable index of manganese status, for example, whole blood, plasma, hair or nails. Although the concentrations of manganese in both plasma and whole blood increase with increasing intake, the whole-blood concentration is 5–l0 times greater than plasma and is therefore more likely to be a sensitive index. Milne *et al.* (1990) suggested whole blood or lymphocytes as indicators of manganese status. It is clear from the studies of Cawte and Florence (1987) and from studies on parenteral feeding that

measurement of whole-blood manganese provides a valid assessment of the uptake of manganese from contaminated foods. Whole-blood manganese can be determined relatively simply by ETA-AAS; sample volumes of only 50–100 µl are required. Good quality control procedures can ensure that the accuracy and precision of these assays is within the range of 3–5%.

2.1.3 MERCURY

Mercury pollution has occurred in the Brazilian Amazon as a consequence of gold-mining (Cleary *et al.,* 1994). Production in this area has been estimated at 12–20 tonnes of gold annually since 1979, with a similar discharge of mercury into the regional ecosystem. Mercury levels in blood and urine high enough to produce clinical symptoms of mercury poisoning have been observed in both mineworkers and in villagers living remotely from mining activities. A particular cause for concern was the high level of mercury in the blood of villagers eating fish caught locally. The mercury concentration in the fish ranged from 9 µg/kg to 2 600 µg/kg fresh weight. Blood and urine samples were collected by a physician at improvised clinics and frozen within 48 hours; mercury was determined using ICP-MS with stringent quality control procedures.

A comparison of blood and urine mercury levels in subjects from Crepuri, a large gold-mining camp and from the village at Jacareacanga, 100 km from the nearest mining camps yields fascinating data on the relative exposure to inorganic mercury and to methyl mercury (organic). Mercury in urine is an appropriate indicator of the former, whereas blood mercury is a biomarker for the ingestion of methyl mercury in fish tissue. The subjects from Crepuri, who would be exposed to inorganic mercury, showed marked elevations in urine mercury, with six individuals having concentrations of ≥ 100 µg/l, a level at which there is a high probability of developing classical signs of mercurialism. Their blood mercury levels were also elevated, with 12 individuals (out of 25) having concentrations of ≥ 20 µg/l which is the upper 95% limit for industrial workers in the UK.

The distribution of blood and urine mercury levels in 25 villagers from Jacareacanga was markedly different from that of the gold-miners. Only one subject had a urine mercury level of more than 100 µg/l and he burned 1 kg of gold, as mercury/gold amalgam, (equivalent to 1 kg of mercury) per week. All of the other villagers studied had urine mercury concentrations of less than 35 µg/l.

However, the incidence of elevated blood mercury levels among these villagers was much higher than among the gold-miners. Most (80%) of the villagers studied had blood mercury concentrations of ≥ 20 µg/l, which is more than the upper limit for industrial workers, and eight out of 25 had blood mercury levels of ≥ 100 µg/l. Four subjects had blood mercury levels that either exceeded or approached the level of 200 µg/l, which may be associated with neurological changes characterised by paresthesia.

These data show clearly that the concentration of mercury in urine is a useful biomarker for exposure to inorganic mercury and that the concentration of mercury in whole blood is a good biomarker for the intake and uptake of methyl mercury from foods.

2.1.4 LEAD

Concentrations of lead in whole blood provide the best index of recent exposure to inorganic lead. However, one difficulty in assessing current environmental lead exposure is the substantial decreases in blood-lead levels over the past decade which have been seen in the USA (Gunter, 1995), South Africa (Grobler *et al.,* l992), Europe (Strömberg *et al.,* 1995) and the UK. The decline in blood-lead is mainly a consequence of the decreased use of lead in petrol, even though there is has also been a decrease in exposure from other sources, such as drinking-water, canned foods, ceramic glazes, cosmetics, decorative paints and industrial processes.

Over the past ten years, the mean/median blood-lead level of non-industrially exposed populations in the UK has fallen by three to four times to about 3 µg/l00 ml; the 95th percentile has halved to about 8–l0 µg/100 ml, which is two and a half to three times lower than the UK action limit. It has been necessary to employ a good internal quality control protocol to minimise temporal changes in analytical bias in order to ensure the validity of these changes.

The measurement of stable lead isotopes in whole blood, teeth and in environmental sources may enable the identification of sources of lead and also apportionment of their relative contributions to body lead. For these investigations to be successful, the different sources of lead need to be isotopically distinct and the increased uptake of a given source needs to produce a measurable change in the

isotopic composition of body lead. In the UK, petrol lead has a distinctively low $^{206}Pb : ^{207}Pb$ ratio of about 1.07, which is significantly different from that in the lead used in old water-supply pipes and present in UK mine deposits (about 1.18). These differences have been exploited to show the relative importance of the sources in different environmental exposure studies in the UK (Alexander *et al.,* 1993; Delves & Campbell, 1993). The power of stable lead isotope analysis is evident from elegant work by Gulson and Wilson (1994), who were able to discern prenatal exposure from lead-mine dusts in sections of deciduous teeth.

2.1.5 CONCLUSIONS

The examples given show that concentrations of trace elements in body tissues and fluids can provide useful indications of environmental exposure. There is, however, scope for improvement. More information could be obtained from a study of the elemental species within a given specimen rather than just the total concentration of all species. Changes in lead isotope ratios in blood in response to changes in uptake may be influenced by the contribution of skeletal lead to blood lead, particularly for adults. An improved detection of these changes can be obtained by measuring all four stable isotopes of lead and not just $^{206}Pb : ^{207}Pb$ ratios.

Notwithstanding these limitations, concentrations of trace elements (and stable isotopes) in body fluids and tissues can be useful biomarkers of excessive intakes and uptakes from environmental sources.

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2.2 CARCINOGENS AND ADDUCTS

PB Farmer and DEG Shuker

MRC Toxicology Unit, University of Leicester

2.2.1 INTRODUCTION

Over the past 20 years or so the development of exquisitely sensitive analytical techniques has enabled the detection and quantitation, in humans, of DNA and protein adducts arising from exposure to carcinogens in the environment. The purpose of this brief overview is to highlight the capabilities and limitations of currently available analytical methodology, and to indicate gaps in knowledge which limit progress in the use of biomarkers in the assessment of human exposure to environmental carcinogens.

2.2.2 METHODS FOR THE MEASUREMENT OF DNA AND PROTEIN ADDUCTS IN HUMANS

DNA ADDUCTS

Much of the early work on modification of DNA by chemical carcinogens made use of radiolabelled compounds, which meant that any DNA adducts already

present were not detected. As analytical methodology improved, it became possible to detect low levels of DNA modification (less than 1 modified base in 10 000 normal bases) arising from *in vitro* or *in vivo* exposure to unlabelled carcinogens. With recent further improvements in a number of areas, DNA adducts can now be detected in small quantities of DNA (less than 1 μg) extracted from small samples of tissue or blood from nominally unexposed humans (Schut & Shiverick, 1992). A distinction can be drawn between two types of techniques that are currently used for the analysis of DNA damage.

Chemical-specific techniques such as immunoassays, mass spectrometry (MS) in combination with gas chromatography (GC) or liquid chromatography (LC), atomic absorbance spectrometry, electrochemical detection (ECD) and competitive DNA repair assays, rely on knowledge of the structure and properties of the particular DNA adducts to be detected. Such techniques are often oriented towards the measurement of DNA damage in situations where the exposure circumstance is well characterised, such as consumption of mycotoxin-contaminated food or food that contains heterocyclic amines as a result of cooking.

Non-specific techniques demand only a minimum requirement, such as the fact that DNA is covalently modified in such a way that it perturbs the function of an enzyme, as in the case of the nuclease P1 version of ^{32}P-postlabelling, or that a detectable property such as radioactivity (in minute quantities) is introduced into DNA, as in the case of accelerator mass spectrometry.

The successful application of DNA adduct analysis depends on a combination of factors, including the analytical sensitivity of the method, the amount of DNA available and the appropriateness of the tissue as a source of DNA. There have been a number of studies on human exposure to methylating agents from medicinal and environmental sources; the various methods used have been summarised and critically evaluated in two recent reviews (Wild, 1990; Bianchini & Wild, 1994).

DNA REPAIR PRODUCTS

Most kinds of DNA damage are efficiently removed by a range of repair enzymes or pathways. Repair pathways that result in excision of the modified base are of particular interest for biomonitoring, since the excised products are often excreted in urine without further metabolism. The use of urinary DNA adducts as markers of carcinogen exposure has been reviewed by Shuker and Farmer (1992). A

number of urinary DNA adducts have been quantified by various methods: 8-hydroxy-2'-deoxyguanosine by high-performance liquid chromatography (HPLC) or immunoaffinity-ECD (Shigenaga *et al.,* 1989; Degan *et al.,* 1991); aflatoxin-*N*7-guanine by immunoaffinity-HPLC-fluorescence (Groopman *et al.,* 1985); 3-alkyladenines by immunoaffinity-GC-MS (Shuker & Bartsch, 1994); and 7-alkylguanines by tandem MS (Cushnir *et al.,* 1990). As urine is generally available in large quantities, the limit of detection of the assays depends only on the sensitivity of the analytical technique. Furthermore, measures of urinary DNA adducts provide an integrated measure of exposure from all sources, both endogenous and exogenous.

PROTEIN ADDUCTS

Adducts of carcinogens with proteins are not normally associated with toxicity or carcinogenicity. The measurement of protein adducts does however possess considerable advantages in comparison to that of DNA adducts, owing to the ready availability of protein from blood samples. Both haemoglobin and to a lesser extent, albumin have been used for biomonitoring. Adducts in proteins are generally stable, in many cases up to the lifetime of the protein, which is about 120 days for haemoglobin; the half-life for albumin is about 20 days.

Unlike the situation for the analysis of DNA adducts, the techniques used for protein adduct analysis are all (reasonably) chemically specific. The analytical methods employed include MS, immunoassay and fluorescence detection (normally after HPLC separation).

The structural evidence obtained to date indicates that the main sites of adduct formation in haemoglobin are (as expected from their nucleophilicity) cysteine, histidine, *N*-terminal amino groups (valine on both the α- and ß-chain of haemoglobin) and the carboxylic groups in aspartic and glutamic acids. Among the carcinogens whose exposure may be monitored with haemoglobin adducts are low molecular weight epoxides (e.g. of ethylene, propylene, 1,3-butadiene, styrene), tobacco-specific nitrosamines (e.g. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNK), acrylamide, acrylonitrile, polycyclic aromatic hydrocarbons (and maybe also benzene), aromatic amines and a variety of other low molecular weight alkylating agents. The main application of albumin adducts for biomonitoring has been for exposure to aflatoxin B_1 (AFB_1), for which the main adducted site is lysine (Sabbioni *et al.,* 1987), and to benzo[*a*]pyrene (Autrup *et al.,* 1991), for which interaction products include aromatic amino acids and histidine (Day *et al.,* 1991).

Dose-response data have been determined for adduct formation by over 20 carcinogens with haemoglobin (Farmer, 1993). In most cases, the lower end of the dose–response curve appears linear, but with high doses of carcinogen some deviations from linearity are observed. The overall conclusion from the dose–response data available is that haemoglobin adducts quantitatively reflect DNA adducts, particularly at low exposure doses. However as demonstrated elegantly by Walker *et al.* (1993), using ethylene oxide, the ratio between haemoglobin and DNA adducts depends on the length of exposure, the interval since exposure, species and tissue.

2.2.3 BIOMARKERS AND EXPOSURES

There are a number of exposures for which biomarkers have been developed and used in studies on human populations. Selected examples are briefly described below.

AFLATOXIN

Several recent studies of human populations with exposure to dietary AFB_1 and elevated liver cancer risk illustrate the value of urinary AFB_1-guanine (AFB_1-Gua) as an informative biomarker. In samples collected from subjects living in Guangxi province, in the People's Republic of China, a good correlation was found between dietary AFB_1 and AFB_1-Gua over a one-week period. A somewhat poorer correlation was observed when daily intake and the following day's excretion were used. Both of these correlations were superior to those obtained when urinary excretion of AFB_1 metabolites, either as a total or individually, was used (Groopman *et al.,* 1992a). A reasonably good correlation was found between dietary AFB_1 intake and total urinary AFB_1 metabolites in subjects from the Gambia, but a better correlation was obtained using AFB_1-Gua. Interestingly, no difference was detected in AFB_1-Gua excretion between hepatitis surface antigen positive and negative carriers for the same dietary intake of AFB_1 (Groopman *et al.,* 1992b; Harris, 1994).

The culmination of much of this work on AFB_1 and risk of hepatocellular carcinoma (HCC) was a recent study carried out in China. A cohort of 18 244 mostly middle-aged (45–64 years) men residing in Shanghai was accrued between

1986 and 1989. In addition to an in-person interview regarding dietary and other past exposures, each subject provided a single void urine sample. After close to 70 000 person-years of follow-up, 55 cases of incident HCC were identified. A nested case–control analysis showed highly significant associations between the presence of urinary aflatoxins (including AFB_1-Gua) serum hepatitis B surface antigen positivity and HCC risk. Risk was especially elevated in individuals who were positive for both of these biomarkers (RR = 59.4) (Ross *et al.,* 1992; Qian *et al.,* 1994). Interestingly, a cohort analysis using the 55 cases of HCC revealed no strong or statistically significant association between HCC risk and dietary aflatoxin consumption as determined from the interview combined with a survey of market foods in the study region. In the authors' words – "the results underline the importance of biomarker measurements in assessing the aflatoxin-HCC association in epidemiological studies" (Qian *et al.,* 1994).

HETEROCYCLIC AROMATIC AMINES

Among the many heterocyclic amines that have been identified in cooked meat, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) has attracted particular attention because it tends to be the most abundant. The major DNA adduct formed by PhIP has been characterised as a C-8 derivative of 2'-deoxyguanosine (Lin *et al.,* 1992) and has been detected in target tissues in experimental animals by ^{32}P-postlabelling (Friesen *et al.,* 1994). PhIP-DNA adducts have recently been detected in human colon samples at levels of three adducts per 10^8 normal nucleotides. It is of particular interest to note that when the positive samples detected by ^{32}P-postlabelling were also analysed by negative ion chemical ionisation (NICI) GC-MS of the derivatised PhIP obtained by alkaline hydrolysis of DNA, clearly positive and quantifiable results were given by this unambiguous and sensitive procedure (Friesen *et al.,* 1994).

MALONDIALDEHYDES

Malondialdehyde (MDA) is the most abundant carbonyl compound and the major mutagenic and carcinogenic product generated by lipid peroxidation (Benamira *et al.,* 1995). The major DNA adduct formed at neutral pH is the highly fluorescent pyrimidopurinone product from 2'-deoxyguanosine (M_1dG). Recent evidence suggests that M_1dG is present in human liver DNA at levels of 5-11 adducts per 10^7 bases (Chaudhary *et al.,* 1994). The analytical method involved the isolation of the pyrimidopurinone guanine base adduct (M_1Gua) from DNA

hydrolysates followed by conversion to a pentafluorobenzyl derivative which was quantified by NICI GC-MS (Chaudhary *et al.,* 1994). The limit of sensitivity of the assay was approximately 2 adducts per 10^8 base pairs with 300 µg of DNA. Malondialdehyde-DNA adducts (including M_1dG) were also detected in normal and tumorous human breast tissues using ^{32}P-postlabelling at levels of 2–200 adducts per 10^9 normal bases (Wang *et al.,* 1995). Previous work had suggested that both rats and humans excreted the base M_1Gua in urine (Hadley & Draper, 1990; Agarwal & Draper, 1992) but this could not be satisfactorily reproduced in another laboratory (Jajoo *et al.,* 1992).

POLYCYCLIC AROMATIC HYDROCARBONS

A number of polycyclic aromatic hydrocarbons (PAHs) have been shown to be carcinogenic. As PAHs are principally formed during combustion of organic matter, their role in carcinogenesis linked to environmental pollution and tobacco smoke has attracted much attention. Using ^{32}P-postlabelling, Phillips *et al.* (1988) showed that there was a dose-dependent increase in 'bulky' adducts in DNA from the lungs of smokers. The same technique has recently been used to demonstrate that PAH adducts were significantly increased in lymphocyte DNA in a population living in a polluted area of Poland during winter months compared to summer months (Perera *et al.,* 1992). Various techniques have been developed for the analysis of PAH-DNA adducts in human tissues including immunochemical assays, ^{32}P-postlabelling assays and HPLC-synchronous fluorescence spectrometry (Alexandrov *et al.*, 1992). A recent development has been the use of a sensitive HPLC fluorescence technique which allows unambiguous quantitation of benzo[*a*]pyrene adducts in human lung DNA (Alexandrov *et al.*, 1992). Total adduct levels determined by ^{32}P-postlabelling ranged from 3 to 13.4 per 10^8 nucleotides, with benzo[*a*]pyrene diol epoxide adducts ranging from non-detectable to 9.9 per 10^8 nucleotides.

AROMATIC AMINES

A number of aromatic amines, including 4-aminobiphenyl, are present in tobacco smoke (IARC, 1986) and this has prompted the development of specific methods to measure human exposure to them. Upon metabolic activation, aromatic amines bind to blood proteins. In particular, thiol groups in haemoglobin were found to be one of the main targets of reactive intermediates and to react with aromatic amine metabolites to give sulphonamide adducts. Treatment of haemoglobin with

alkali causes the adducts to decompose and the parent amine is released and subsequently converted into an *N*-perfluoroacyl derivative, which can be quantified with great sensitivity by GC-NICI-MS. 4-Aminobiphenyl adducts were found in haemoglobin of smokers and non-smokers; smokers had significantly higher levels than non-smokers (Bryant *et al.*, 1988). In a study of smokers attending a clinic aimed at helping them to stop smoking, 4-aminobiphenyl adducts decreased in the majority of smokers who succeeded in stopping smoking, with a time course that largely reflected the turnover of erythrocytes. Some subjects showed little change in 4-aminobiphenyl levels; it was shown that these subjects had failed to stop smoking. (Maclure *et al.*, 1990).

2.2.4 GAPS IN KNOWLEDGE

STABILITY OF ADDUCTS DURING LONG-TERM STORAGE

One of the most informative kinds of epidemiological inquiry is the case–control study nested in a cohort for whose members there is good information about exposure to toxic agents prior to the onset of disease. This requires collection of biological samples at the time of recruitment of subjects into the cohort and storage of the samples until such time as the study is carried out. The stability of DNA and protein adducts under conditions of long-term storage (several years) is still a largely unexplored area.

IDENTIFICATION OF ADDUCTS DETECTED BY 'SCREENING' PROCEDURES

Techniques such as the ^{32}P-postlabelling procedure are capable of detecting and quantifying DNA damage without the necessity for detailed *a priori* knowledge of the structure of the adduct. This is both an advantage and a disadvantage. In the case of human exposure to complex environmental mixtures of toxic substances, screening procedures are capable of alerting researchers to a potential hazard. However, in order to make decisions about action to be taken to reduce exposure, some knowledge about the identity of the active components would obviously be useful. At present the exquisite sensitivity of the ^{32}P-postlabelling procedure means that there is no independent means of identifying the structure of the DNA adducts (in many cases this would also lead to the identification of the structure

of the agent responsible for the damage). There is also no general procedure for the detection of protein adducts. In view of the relative ease with which large quantities of blood proteins can be obtained, a reliable method for identifying protein adducts would have great value.

RELATION BETWEEN ADDUCTS IN SURROGATE AND TARGET TISSUE

The relation between levels of adducts in surrogate tissues (blood and readily obtained epithelial tissues) and target internal organs has been established for only a few carcinogens (for example, for ethylene oxide by Walker *et al.,* 1993, and for dimethylnitrosamine by Montesano *et al.,* 1992 and references therein). It would appear that this relation is characteristic for each carcinogen. This would indicate that a large amount of experimental work would be required to undertake risk assessment for carcinogens of interest. If the major interest is the use of adducts as markers of exposure, then there is less requirement for this relation to be precisely defined.

RELATION BETWEEN ADDUCTS AND BIOLOGICAL END-POINTS

This is an area of major interest, particularly in the realm of chemical carcinogenesis. As knowledge of the mechanisms of cancer increases, there is an expectation that markers of early molecular events will provide a better indication of the risk of development of cancer. At present most adduct measurements are probably only better measures of exposure than those obtained by traditional methods (questionnaires or external exposure measurements). Most current assays for DNA adducts measure only average levels of adducts in total genomic DNA. Some attempts have been made to detect adducts in defined sequences (such as tumour suppressor genes) or to use immunoaffinity techniques to purify selectively and amplify gene sequences that contain particular adducts (Hochleitner *et al.,* 1991).

SIGNIFICANCE AND ROLE OF BACKGROUND LEVELS OF ADDUCTS

As the sensitivity and specificity of analytical methods for DNA and protein adducts has increased, it has been observed that there are background levels of

adducts in subjects who would otherwise be classified as unexposed. This can vary from adducts such as 8-hydroxydeoxyguanosine in DNA, which is considered to be largely endogenous in origin, to 4-aminobiphenyl-haemoglobin adducts, which have been detected in all subjects, irrespective of their smoking status. A key question, therefore, for low-level environmental exposures is whether they add significantly to the existing burden of DNA and protein damage and, if they do, whether the increase is manifested in terms of overall risk.

IMPORTANCE OF POLYMORPHISMS IN CARCINOGEN-METABOLISING ENZYME

There is great interest in the role of individual susceptibility to low-level carcinogen exposure which is mediated through genetic polymorphisms in the activity of carcinogen-metabolising enzymes. This is clearly highly relevant if the only means of exposure assessment is some external marker of exposure. In this case the interindividual variation in carcinogen metabolism resulting in activation or detoxification might be expected to result in large differences in the biologically relevant internal dose among subjects exposed to similar doses. A particularly well characterised example is the aromatic amine *N*-acetylator polymorphism which appears to be associated with higher risks of bladder cancer in individuals exposed to aromatic amines in cigarette smoke or at the workplace (Vineis *et al.,* 1990 and references therein).

VALIDATION OF THE USE OF CARCINOGEN ADDUCTS IN CASE–CONTROL STUDIES

There are few examples of studies where biomarkers of carcinogen exposure have been examined in the light of the disease outcome. The use of various biomarkers of low-level aflatoxin exposure to classify exposure proved particularly powerful in the case of aflatoxin and HCC described above. The success of this study is encouraging, in that one of the key arguments for the use of biomarkers in the measurement of internal dose, that is, a more rigorous classification of exposure level which takes account of individual uptake, metabolism and target site modification, was strongly vindicated. It is nonetheless worthwhile pointing out that aflatoxin adducts are very characteristic and are relatively free of the background problems described above.

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2.3 SOLVENTS AND GASES

K Wilson

Health and Safety Laboratory, Sheffield

2.3.1 INTRODUCTION

This review identifies some of the essential elements that should be considered when determining the potential role of biomarkers, particularly for solvents and gases, in human environmental exposure; possible biomarkers for substances of environmental interest are also identified. The part that physiologically based pharmacokinetic (PBPK) modelling may play in environmental exposure assessment and health risk assessment is also reviewed briefly.

The major air pollutants are generally considered to be benzene, ozone, carbon monoxide, sulphur dioxide, nitrogen dioxide and butadiene. To this list can be added a variety of volatile organic compounds (VOCs) and related substances such as polycyclic aromatic hydrocarbons (PAHs).

2.3.2 CRITERIA FOR SELECTING A BIOMARKER METHOD

Ideally, the selected biomarker should:

- be specific (biologically and analytically)
- be measured in samples obtained by non-invasive methods (for ease of collection)
- be determined using a robust analytical method
- be amenable to quality assurance (longitudinal) procedures
- be suitable for screening (through put 20 samples per day; relatively inexpensive)
- have well established kinetics (timing of sample)
- produce readily interpretable results.

Other considerations include:

- analytical problems (sensitivity, background contamination)
- knowledge about confounders, interactions, interferences and limitations
- establishing whether results should be corrected (e.g. urinary creatinine, body fat or carbon monoxide)
- defining exposure route, e.g. dermal, ingestion, inhalation
- defining exposure groups e.g. smokers, urban dwellers, etc.
- identifying vulnerable groups e.g. pregnant women and new mothers, children, elderly etc.

Some of the factors, such as alcohol, drugs and smoking, which are known to affect the levels of biomarkers have been discussed by Alessio *et al*. (1995). Woollen *et al*. (1990) have shown that the predictive value of breath analysis can be improved if the results are normalised to body fat. There is some debate about the value of creatinine correction of urinary results (Boeniger *et al.,* 1993). Sampling, quality assurance, interpretation and other issues, such as indoor *versus* outdoor air quality, emission sources and delivery are discussed by Heinzow and McLean (1994) and Wallace (1995). Nielsen *et al*. (1995) have reviewed biomarkers, respiratory tract pollutants (including ozone, nitrogen dioxide) and groups at increased risk. A substantial number of relevant papers appear in the proceedings of the International Symposium on Human Health and Environment, Italy (Mutti *et al.,* 1995).

2.3.3 SUBSTANCES FOR WHICH EXPOSURE BIOMARKERS HAVE BEEN IDENTIFIED

The development of most exposure biomarkers has emerged from within the occupational health field. The list in Table 2.3.1 gives an overview of the type of substances for which analytical methods are available based on parent compounds or metabolites in blood, urine or breath.

2.3.1 Substances for which biomarkers of exposure have been established

Substance	Biomarker Blood	Urine	Breath
Hydrocarbons			
Acetone		Acetone	
Benzene	Benzene	*trans,trans*-Muconic acid, S-Phenylmercapturic acid	Benzene
Butoxyethanol		Butoxyacetic acid	
Ethoxyethanol		Ethoxyacetic acid	
Methoxyethanol		Methoxyacetic acid	
Ethylbenzene		Mandelic acid	
Cyclohexane		Cyclohexanol	
Furfural		Furoic acid	
Hexane		Hexanedione	

Substance	Biomarker Blood	Urine	Breath
Methanol		Methanol	
Methyl butyl ketone		Hexanedione	
Methyl ethyl ketone		Methyl ethyl ketone	
Phenol		Phenol	
Methyl *t* butyl ether	Methyl *t* butyl ether		
Propanol		Acetone	
Styrene		Mandelic acid	
Toluene	Toluene		
Xylene		Methylhippuric acid	
Halogenated hydrocarbons			
Carbon tetrachloride	Carbon tetrachloride		
Chlorobenzene		Chlorocatechol	
Chloroform	Chloroform		Chloroform
Chlorophenol		Tri- and tetra-chlorophenols	
Chlorophenoxy acids		2,4- or 2,4,5-Chlorophenoxy acetic acid	
Dichloromethane	Carboxyhaemoglobin		Carbon monoxide
Halothane		Trifluororoacetic acid	
Hexachlorobenzene	Hexachlorobenzene		
Methylbromide	Bromine		
Pentachlorophenol		Pentachlorophenol	
Polychlorinated biphenyls	Polychlorinated biphenyls		
Tetrachloroethylene	Tetrachloroethylene		Tetrachloroethylene
Trichloroethane	Trichloroethane		
Trichloroethylene		Trichloroacetic acid	
Trichlorotrifluoroethane		Trichlorotrifluoroethane	
Substances containing nitrogen, sulphur etc.			
Acrylonitrile		Thiocyanate	
Aniline		Aminophenol	
Carbon disulphide		Thiothiazolidine-4-carboxylic acid	
Carbon monoxide	Carboxyhaemoglobin		Carbon monoxide
Dimethylformamide		*N*-Methylformamide	
Dinitro-o-cresol		Dinitro-*o*-cresol	
Methylenebis(*o*-chloroaniline)		Methylenebis(*o*-chloroaniline)	
Methylenedianiline		Methylenedianiline	

Substance	Biomarker Blood	Urine	Breath
Nitrobenzene		Nitrophenol	
Organophosphates		Dialkylphosphates	
Parathion		Nitrophenol	
Polycyclic aromatic hydrocarbons		Hydroxypyrene	

2.3.4 BIOMARKERS IN ENVIRONMENTAL EXPOSURE ASSESSMENT

A few selected substances (benzene, trichloroethylene, chloroform, perchloroethylene, butoxyethanol, VOCs, PAHs carbon monoxide (CO) and ozone and nitrogen oxides) for which biomarkers have been used to assess environmental exposure assessment are reviewed below (see also Table 2.3.2).

BENZENE

Most published methods seem to be able to discriminate between smokers and non-smokers. Brugnone *et al.* (1992) have reported reference values for blood benzene in the general population. The most promising non-invasive method would appear to be urinary *trans,trans*-muconic acid or S-phenylmercapturic acid.

TRICHLOROETHYLENE

Trichloroacetic acid in blood and urine is readily measured in urban populations where the drinking-water contains trichloroethylene (Skender *et al*., 1994).

CHLOROFORM

Chloroform has been found in the blood, breath and urine of people using swimming pools (Aggazzotti *et al.,* 1993; Cammann & Hubnet, 1995).

Table 2.3.2 The application of biomarkers to environmental exposure assessment

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Benzene						
Blood benzene	Rural		200 ng/l	7–1003 ng/l	Purge/trap GC-MS	Brugnone *et al.*,
	Urban		296 ng/l	7–2241 ng/l	(15 ng/l)	1992
	Non-smokers		176 ng/l	80–300 ng/l	Purge/trap FID	Angerer *et al.*,
	Smokers[a]		211 ng/l	130–430 ng/l	(80 ng/l)	1991
	Smokers		365 ng/l	170–730 ng/l		
	Non-smokers		110 ng/l	50–218 ng/l	Headspace PID	Kok & Ong, 1994
	Smokers		328 ng/l	81–628 ng/l	(50 ng/l)	
	Exposure to petrol fumes and automotive exhaust	41 µg/m^3	290 ng/l	120–1970 ng/l	GC-MS (30 ng/l)	Mannino *et al.*, 1995
Urine benzene	Non-smokers		116 ng/l	50–344 ng/l	Headspace PID	Kok & Ong 1994
	Smokers		404 ng/l	110–1450 ng/l	(40 ng/l)	
Breath benzene	Non-smokers		6.2 µg/m^3	± 7.5 µg/m^3	Adsorption tube-GC	Ljungkvist &
	Smokers		14.6 µg/m^3	± 8.8 µg/m^3	(0.5 µg/m^3)	Nordlinder, 1995
Urine *trans, trans*-muconic acid	Non-smokers		0.05 mg/g creatinine	0.03–0.08 mg/g creatinine	GC-MS (10 µg/l)	Ruppert *et al.*, 1995
	Smokers		0.09 mg/g creatinine	0.04–0.14 mg/g creatinine		
	Exposed		1.7 mg/g creatinine		HPLC-UV (10 µg/l)	Boogaard & van Sittert, 1995
	Non-smokers (controls)		0.037 mg/g creatinine			
	Smokers (controls)		0.058 mg/g creatinine			

Table 2.3.2 (cont.)

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Benzene (cont.)						
Urine *trans, trans*-muconic acid	Non-smokers		0.14 mg/g creatinine	0.01–0.29 mg/g creatinine	HPLC-UV (25 µg/l)	Lee *et al.*, 1993
	Smokers		0.19 mg/g creatinine	0.06–0.43 mg/g creatinine		
	Exposed occupationally	< 1ppm [3.2 mg/m^3]	0.36 mg/g creatinine	0.09–1.56 mg/g creatinine	HPLC-UV	Ong *et al.*, 1995
	"	1–5ppm [3.2–6 mg/m^3]	4.59 mg/g creatinine	1.49–24.87 mg/g creatinine		
	Non-exposed		0.11 mg/g creatinine	0.01–0.29 mg/g creatinine		
Urine S-phenylmercapturic acid	Exposed		47 µg/g[b] creatinine		(1 µg/l)	Boogaard & van Sittert, 1995
	Non-smokers (controls)		1.99 µg/g creatinine			
	Smokers (controls)		3.61 µg/g creatinine			
Urine catechol	Exposed occupationally	<1ppm[3.2 mg/m^3]	1.71 mg/g creatinine	0.47–10.86 mg/g creatinine	HPLC-FL	Ong *et al.*, 1995
	Unexposed		1.52 mg/g creatinine	0.25–4.78 mg/g creatinine		
Urine hydroquinone	Exposed occupationally	<1ppm[3.2 mg/m^3]	0.64 mg/g creatinine	0.16–1.58 mg/g creatinine	"	"
	Unexposed		0.32 mg/g creatinine	0.04–1.9 mg/g creatinine		

Table 2.3.2 (cont.)

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Trichloroethylene						
Blood trichloroethylene	Urban population	Drinking water 0.05–22.9 µg/l		0.015–0.09 µg/l	Headspace GC (0.015 µg/l)	Skender *et al.*, 1994
Plasma trichloroacetic acid	"	"		8.6–148 µg/l	" (0.5 µg/l)	"
Urine trichloroacetic acid	"	"		1.67–102 µg/24h	" (0.5 µg/l)	"
Chloroform						
Breath chloroform	People attending indoor swimming pools	Air 410–2345 mmol/m^3 [49–280 µg/m^3],	788 nmol/m^3 [94 µg/m^3]	117–2614 nmol/m^3 [14–312 µg/m^3]	Glass tubes[c] GC (8 nmol/m^3) [1 µg/m^3]	Aggazzotti *et al.*, 1993
	Non-exposed	Water 159–787 nmol/ l [19–94 µg/l]	100 nmol/m^3 [12 µg/m^3]			
Blood chloroform	Competitive swimmers			1.14–5.23 µg/l	Headspace GC-ECD	Cammann & Hubner, 1995
	Normal swimmers			0.56–1.65 µg/l		
	Attendants			0.13–2.45 µg/l		
Urine chloroform	Swimmers			~ 0.2–2.0 µg/l	"	"

Table 2.3.2 (cont.)

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Perchloroethylene						
Breath perchloroethylene	Unexposed homes	3 µg/m^3	4 µg/m^3	1–16 µg/m^3	Glass tubes[c] GC-ECD	Aggazzotti *et al.*, 1994
	Dry-cleaners' homes	500 µg/m^3				
	family		321 µg/m^3	30–900 µg/m^3		
	dry-cleaners		8666 µg/m^3	800–39200 µg/m^3		
Blood perchloroethylene	Urban population	Drinking water 0.21–7.8 µg/l	~0.07 µg/l	0.01–0.24 µg/l	Headspace GC (0.01 µg/l)	Skender *et al.*, 1994
Butoxyethanol						
Blood butoxyethanol	Volunteers				GC-ECD	Johanson & Boman 1991
	inhalation	2 mmol/m^3 [240 mg/m^3]	2.9 µmol/l [0.34 mg/m^3]			
	skin		7.4 µmol/l [0.87 mg/m^3]			
	Unexposed volunteers				GC-MS-NICI (1.5 ng/g ^{13}C)	Bormett *et al.*, 1995
Blood butoxyacetic acid	"			2.5–12 ng/g	" (0.4 ng/g ^{13}C)	"

Table 2.3.2 (cont.)

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Volatile organic compounds						
Blood VOC:	Exposure to petrol fumes and automotive exhaust				GC-MS	Mannino *et al.*, 1995
Methyl *t*-butyl ether	"	1500 µg/m^3	0.24 µg/l	ND–1.5 µg/l	" (0.5 µg/l)	"
t-Butyl alcohol	"		2.24 µg/l	0.69–13.8 µg/l	" (0.5 µg/l)	"
Ethyl benzene	"	54 µg/m^3	0.23 µg/l	0.04–3.03 µg/l	" (0.02 µg/l)	"
o-Xylene	"	61 µg/m^3	0.32 µg/l	0.07–2.01 µg/l	" (0.04 µg/l)	"
m- and *p*-Xylene	"	230 µg/m^3	0.95 µg/l	0.1–9.78 µg/l	" (0.03 µg/l)	"
Toluene	"	240 µg/m^3	1.07 µg/l	0.14–5.43 µg/l	" (0.09 µg/l)	"
Trichloroethane	"	11 µg/m^3	0.23 µg/l	ND–310 µg/l	" (0.09 µg/l)	"
Perchloroethylene	"	3 µg/m^3	0.12 µg/l	ND–150 µg/l	" (0.03 µg/l)	"
Variety of substances in blood, urine and human milk				0.01–1.28 µg/l	Purge/trap GC-MS (<0.1 µg/l)	Dunemann & Hajimiragha, 1993

Table 2.3.2 (cont.)

Biomarker	Cohort	Environ. level	Median/mean	Range	Method (Detection limit)	Reference
Polycyclic aromatic hydrocarbons						
Urine 1-Hydroxypyrene	Windsurfers					Jongeneelen, 1994
	Surfing days		0.32 µmol/mol creatinine	0.16–0.81 µmol/mol creatinine		
	Non-surfing days		0.11 µmol/mol creatinine	0.05–0.16 µmol/mol creatinine		
	Netherlands adults		0.26 µmol/mol creatinine			"
	Polish children (heavy industry)		0.46 µmol/mol creatinine	0.09–6.99 µmol/mol creatinine		
	Unexposed		0.38 µg/l	ND–1.7[e] µg/l	HPLC-FL (0.1 µg/l)	Whiton *et al.*, 1995
Urine 1-hydroxypyrene glucuronide [d]		Estimated pyrene exposure: 0.5–50 nmol/day [0.1–10 mg/day]		0.06–17 nmol/l	HPLC-FL GCMS	Strickland *et al.*, 1994

ECD, electron capture detection; ETS, environmental tobacco smoke; FID, flame ionisation detection; FL, fluorescence detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; ND, not detectable; NICI, negative ion chemical ionisation; PID, photoionisation detection; UV, ultraviolet detection VOC, volatile organic compound

[a] smokers abstinent for at least one day; [b] calculated average concentration following occupational exposure to 8h TWA of 3.2 mg/m^3 (1ppm); [c] for alveolar breath sampling; [d] research method; [e] exceeded calibration range

PERCHLOROETHYLENE

Perchloroethylene has been measured in the breath and blood of people living in or near dry-cleaning establishments (Aggazzotti *et al.,* 1994; Skender *et al.,* 1994).

BUTOXYETHANOL

Dermal penetration of glycol ethers may be a more significant route of exposure than inhalation. A published gas chromatography-mass spectrometry (GC-MS) negative ion chemical ionisation (NICI) method is highly sensitive and specific and appears to be capable of measuring environmental levels. (Johanson & Bowman, 1991; Bormett *et al.,* 1995)

VOLATILE ORGANIC COMPOUNDS

A number of VOCs are detectable in blood and urine using GC-MS with purge and trap. (Dunemann & Hajimiragh, 1993; Mannino *et al.,* 1995)

POLYCYCLIC AROMATIC HYDROCARBONS

1-Hydroxypyrene and related metabolites have been used as markers of PAH exposure. The data suggest that groups receiving different levels of environmental exposure can be easily identified (Jongeneelan, 1994; Strickland *et al.,* 1994; Whiton *et al.,* 1995).

CARBON MONOXIDE

Breath levels of CO have been shown to rise in non-smoking indoor hockey players (Lee K. *et al.,* 1994). A good correlation between breath CO and carboxyhaemoglobin (COHb) using diode laser spectrometry has been shown in non-smoking volunteers exposed to low levels of CO (Lee, P.S. *et al.,* 1994). A rapid microanalysis method for measuring CO in blood by headspace GC with thermal conductivity detection has been reported (Van Dam & Daeneus, 1994).

OZONE

It has been suggested that a cellular response using nasal lavage can be used to measure the acute inflammatory effect of low level ozone exposure. (Graham & Koren, 1990). Devlin, (1993) speculates on the possibility of defining a range of proteins or mRNAs which are induced by ozone or other pollutants.

NITROGEN OXIDES

Ewetz (1993) has reviewed the absorption and metabolic fate of nitrogen oxides. Inhaled nitrogen dioxide enters the blood as nitrite and is excreted in urine as nitrate. Nitrogen oxide enters the blood as nitric oxide and appears in the urine as nitrate. There also appears to be an association between nitrogen dioxide in urban air and the formation of *N*-nitroso compounds.

2.3.5 APPROPRIATE METHODS

The most popular routine analytical techniques in use are:

- GC with sensitive and specific detectors such as flame photometric, electron capture, photoionisation, MS with electron impact, NICI, etc.
- High-performance liquid chromatography with ultraviolet or fluorescence detectors
- Techniques for measuring volatile components in biological fluids including headspace analysis and purge and trap followed by GC-MS; background contamination in air and reagents can cause significant problems
- Breath analysis, which has made some progress over the past few years; methods are now available for collecting breath onto suitable adsorbents (Ljungkvist & Nordlinder, 1995); respiratory MS has been used to screen working populations (Drummond *et al.,* 1988).

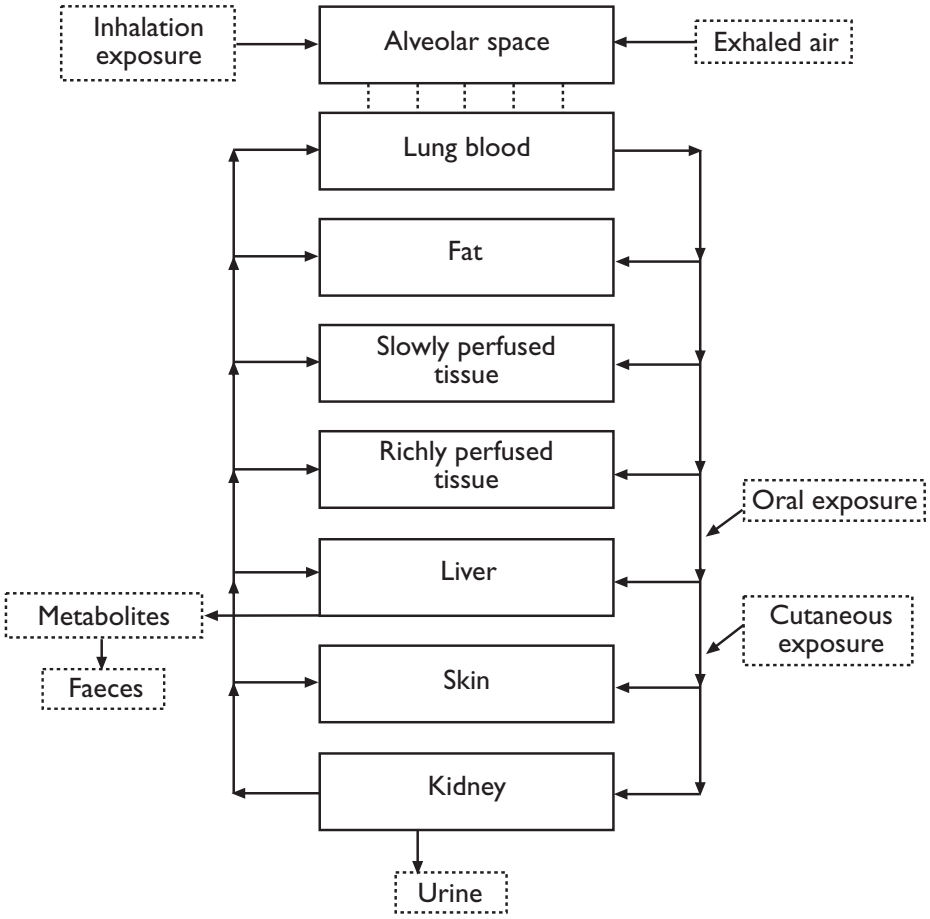
2.3.6 THE USE OF PHARMACOKINETIC MODELS IN BIOMONITORING

There is an extensive literature on the development and use of pharmacokinetic models. Two main approaches have been used:

- data-based compartment modelling, which relies on time-course data fitted to a mathematical model.
- the more sophisticated PBPK model based on compartments that correspond to anatomical structures; the PBPK model is the most popular and has the generic form illustrated in Figure 2.3.1.

These models are defined with respect to volume, blood flow, partitioning, metabolism, etc., and require a set of averaged physiological data and physicochemical parameters. They have been used to model the behaviour of solvent mixtures (Tardif *et al.,* 1995), ethanol/solvent interactions and other confounding factors (Sato, 1993), work schedules (Saltzman, 1988) and exposure scenarios (Laparé *et al.,* 1993), and different exposure routes (Shyr *et al.,* 1993), and in setting exposure limits (Droz & Fiserova-Bergerova, 1992). Droz (1992) has also identified the extent of biological variability and Dallas *et al.* (1995) have studied species differences. Predictions have been made on the dose-dependent kinetics of styrene exposure in humans (Lof & Johanson, 1993). A model has been developed linking indoor air perchloroethylene to risk assessment (Bogen & McKone, 1988). Other models have included lactational transfer and infant exposure to perchloroethylene (Schreiber, 1993; Byczkowski & Fisher, 1994). A PBPK model was modified to account for acetone uptake in the mucous layer of the respiratory tract (Kumagai & Matsunaga, 1995). Two international meetings have been held on current developments on modelling and health risk assessment (Johanson & Droz, 1992; Wilson *et al.,* 1995).

Figure 2.3.1 The use of pharmacokinetic models in biomonitoring



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2.4 BIOMARKERS FOR EXPOSURE (AND EFFECT) ASSESSMENT OF DIOXINS AND PCBs

A Brouwer

Department of Toxicology, Agricultural University Wageningen, The Netherlands

2.4.1 INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are classes of persistent environmental pollutants, which may exert prototypical, pleiotropic dioxin toxicity. These chemicals are ubiquitous in the environment as complex mixtures, which theoretically may contain more than 400 different isomers. Owing to their lipophilic nature and extreme biostability they show a high tendency to bioaccumulate in food-chains. However, the congeneric pattern of PCDDs, PCDFs and PCBs in sediments, soil and other primary sources differs considerably to the patterns found in biotic samples from various species. In general, the more persistent 2,3,7,8-substituted PCDD and PCDF congeners are found at higher concentrations in top predators and other species at the top of food-chains; these congeners are also the most toxic. For PCBs, congener-dependent bioaccumulation is more complex, but again many of the persistent PCBs are also very toxic.

The complex nature of PCDD, PCDF and PCB mixtures, the wide difference in species-specific congener patterns, which may exert different toxic responses, and the extreme differences in species-dependent sensitivity to the toxic actions of these chemicals present serious problems for exposure and risk assessment and for extrapolation of results from, for example, laboratory animals to wildlife species and humans. Several attempts have been made to overcome these problems; for example, the so called 'toxic equivalency concept' allows the conversion of a complex mixture of congeners into one sum parameter, the dioxin toxic equivalency (Safe, 1994). In addition, several biomarker systems have been developed that may reflect the sum toxicity of complex PCDD, PCDF and PCB mixtures. These aspects will be addressed in this review.

2.4.2 MECHANISM OF DIOXIN TOXICITY

PCDDs, PCDFs and PCBs cause a wide variety of toxic effects, including liver toxicity, immune suppression, dermal lesions (hyperkeratosis), reproductive toxicity, teratogenicity, carcinogenicity and developmental neurotoxicity (McConnell, 1989; Safe, 1994). In addition, many pathways of intermediary metabolism are affected, resulting in hormonal disturbances (thyroid and sex hormones), alterations in vitamin (vitamins A and K) and lipid metabolism, porphyrinogenic actions, and induction of several biotransformation enzymes, such as cytochrome P450 isozymes (Safe, 1994).

Most of these effects are caused by a receptor-mediated activation process. PCDD, PCDF and PCB congeners, with *meta* and *para* chlorine substitution, and no or limited *ortho* substitution, show a high binding affinity for a cytosolic receptor protein, the aryl hydrocarbon receptor (AhR; Poland & Knutson, 1982). The AhR undergoes an activation process following ligand binding, translocates into the nucleus and binds as a heterodimeric complex with another protein, the aryl hydrocarbon nuclear translocator (ARNT), to a selective nucleotide sequence, the dioxin responsive enhancer (DRE; Whitlock, 1993). The DRE sequences are located upstream of genes that show a dioxin-dependent increase in expression of transcribed products following ARNT/AhR-ligand complex binding. The most investigated gene whose expression is under control of the AhR is the CYP1A1 gene. The translated product from this gene, the cytochrome

P4501A1 isozyme, can be measured by the conversion of the model substrate ethoxyresorufin into the fluorescent resorufin (the ethoxyresorufin-*o*-deethylase (EROD) assay). There are many other genes under the control of the AhR-signal transduction pathway, which may be involved in the various toxic end-points induced by PCDD, PCDF and PCB congeners.

2.4.3 THE TOXIC EQUIVALENCY FACTOR (TEF) CONCEPT

Many studies have indicated that the various individual 2,3,7,8-substituted PCDD and PCDF congeners, and the non-*ortho* and, to a lesser extent, mono-*ortho* substituted PCB congeners use the same AhR-signal transduction pathway to exert many of their toxic and biochemical effects. The binding affinity of the various congeners to the AhR correlates well with their potency to induce, for example, EROD activity both *in vitro* and *in vivo* (Safe, 1994). It is this common AhR-mediated mechanism of action that forms the basis of the TEF concept, which allows the summation of dioxin-like congeners within a mixture. Individual toxic potencies are expressed as the TEF factor (the fractional toxicity of the congener relative to the most toxic congener, 2,3,7,8-TCDD) multiplied by the mass quantity of the congener in the mixture. The TEF concept is nowadays widely used for monitoring and risk assessment purposes.

There are however several disadvantages to this approach. Firstly, the TEF concept assumes that the interactive effects of individual congeners in a mixture are additive; however, there are several *in vitro* and *in vivo* studies indicating that PCBs in particular show antagonistic interactive effects (Davis & Safe, 1990). Secondly, the toxicokinetics of the individual congeners may differ considerably *in vivo*, which is not taken into account in the TEF concept. Thirdly, the TEF concept assumes that all toxic end-points of the various congeners are solely mediated *via* the AhR signalling pathway; this appears not to be the case for some toxic effects, like developmental neurotoxicity and carcinogenicity.

In contrast, the TEF concept is based on probably the widest set of data on structure-activity and structure-toxicity relationships for a multitude of individual congeners, toxic end-points and species available for any class of environmental pollutants. Therefore, until alternative approaches or adaptations are available

and acceptable, the TEF concept, by providing a means of estimating the total toxic potency of the mixture, is a helpful interim approach to the interpretation of complex gas chromatography-mass spectrometry (GC-MS) patterns of the various PCDD, PCDF and PCB congeners analysed in environmental and biotic samples.

2.4.4 BIOMARKERS FOR USE IN ORGANOHALOGEN EXPOSURE (AND EFFECT) ASSESSMENT

Several biomarker assay systems for organohalogen exposure (and effect) assessment have been developed over the last ten years; these can be divided into two categories:

- biomarkers directly based on parts of the AhR signalling pathway, such as induction of cytochrome P4501A1 activity (EROD) and the recently developed recombinant cell lines containing AhR-linked reporter gene constructs

- biomarkers indirectly associated with activation of the AhR signalling pathway, such as the production of organohalogen metabolites which compete with natural hormones for receptor binding and function.

The AhR-mediated induction of the cytochrome P4501A1 enzyme and its associated EROD activity is one of the most widely studied biochemical effects of persistent organohalogens, like PCBs, PCDDs and PCDFs. It has therefore been widely used as a biomarker for assessment of exposure to these categories of environmental pollutants. The EROD induction biomarker is used in *in vivo* as well as *in vitro* studies to determine the AhR-mediated toxic potency of both individual and complex mixtures of organohalogens present in environmental and biotic samples. This EROD induction assay system has been proven to be one of the most sensitive biochemical responses of dioxin-like chemicals in laboratory and wildlife species (Safe, 1994; Murk *et al.*, 1994; Van den Berg *et al.*, 1994). However, in some species, like the flatfish *Platichthys flesus*, the EROD induction response caused by, for example, Aroclor 1254, a commercial PCB mixture, is very

low, while a good induction may be observed for 2,3,7,8-TCDD. It appears that this may be caused by the presence of antagonists in the PCB mixture that actually inhibit EROD, the enzymatic activity of cytochrome P450, but not its gene expression (Hahn & Stegeman, 1994). Therefore, the EROD induction data should be interpreted with care and CYP1A1 mRNA levels should also be analysed.

Nowadays several hepatoma cell lines, such as the mouse Hepa-1c1c7 and rat H4IIe cell lines, are available for *in vitro* analysis of the AhR-dependent induction of EROD activity in multi-well plates by both individual congeners and complex mixtures of organohalogens. In addition, a novel approach is to generate, *via* recombinant DNA technology, cell lines which have been stably transfected with AhR-linked reporter gene constructs for biomarker purposes. One example is the chemically activated luciferase gene expression (CALUX) system developed recently (Aarts *et al*., 1993; Garrison *et al*., 1996). In these assays extremely small quantities of organohalogens or extracts are added to the culture media of CALUX cells, which will than start to produce the luciferase enzyme *via* an AhR-dependent pathway. This system appears to have superior sensitivity (around 10 femtomoles) and ease of performance (short exposure times in 96-well plates, with analysis by microplate readers). This CALUX biomarker is now in the validation phase and shows a good correlation with GC-MS data on the presence of dioxin-like chemicals and activities in various matrices, such as human milk, cow's milk, river sediments and human blood samples. An additional advantage is that these systems will allow the identification of hitherto 'unknown' chemicals showing AhR-agonistic, or antagonistic activities.

One consequence of AhR-mediated activation of CYP1A1 is the formation of metabolites, either from the parent compound acting as the inducer, or from other, less biostable contaminants which may also be present in the exposure mixture. These metabolites, although usually functioning as intermediates in elimination pathways may however exert their own specific toxic effects, different from the parent compounds from which they derive. For example, induction of CYP1A1 has been shown to be associated with an increased formation of carcinogenic bay region intermediates of PAHs and therefore may express co-carcinogenic activities (Dipple *et al*., 1984; Yang *et al*., 1985). Another example is the formation of phenolic PCB metabolites as a consequence of CYP1A1 induction (Ishida *et al*., 1991; Morse *et al*., 1995). These phenolic PCB metabolites have been shown to cause a number of biochemical and toxic effects. In particular, they were found to be strong competitive inhibitors of thyroid hormone by binding to its plasma transport protein, transthyretin (Lans *et al*., 1993). In addition, they have been

shown to bind to the oestrogen receptor and have been reported to act, either as agonist or antagonist in the oestrogen-signal transduction pathway (Korach *et al.*, 1987). Biomarkers have recently been developed for determining both the thyroidogenic and oestrogenic disruption potential of phenolic organohalogens and related environmental contaminants.

2.4.5 CONCLUSIONS

Several biomarker assay systems have been developed for assessment of exposure to persistent organohalogens. These systems are based, either directly or indirectly, on the mechanism of action of the classes of chemicals under investigation. This allows the use of the biomarkers as sum parameters for the organohalogens and possible other 'unknown' environmental contaminants acting through the same signal transduction pathway. The results of these biomarker assays not only indicate the level of exposure (exposure assessment) to chemicals, but also reflect a measure of the toxic responses that may be caused by the chemicals and may therefore also be helpful in effect assessment.

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2.5 BIOMARKERS OF EFFECT AND SUSCEPTIBILITY

H Mason

Health and Safety Laboratory, Sheffield

2.5.1 INTRODUCTION

There are generally considered to be three types of biomarker, namely of exposure, effect and susceptibility (Schulte, 1991). A biomarker of effect has been defined as a measurable reversible biochemical change caused by the absorption of the substance. Ideally the degree of change is below that associated with toxic injury or known irreversible pathological effect. A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an individual to respond to the challenge of exposure to a specific agent(s).

In the field of occupational health, where exposures may be relatively high, biomarkers of effect have been shown to provide the benefits of early detection of preclinical disease with the possibility of subsequent ameliorating intervention. The use of biomarkers of susceptibility, in terms of being able to identify individuals at risk, has raised substantial debate about their ethical implications.

This short report gives some examples of the use of biomarkers of effect and susceptibility in the assessment of human environmental exposure rather than as risk indicators of ill-health. It concentrates on non-genotoxic end-points.

2.5.2 BIOMARKERS OF EFFECT

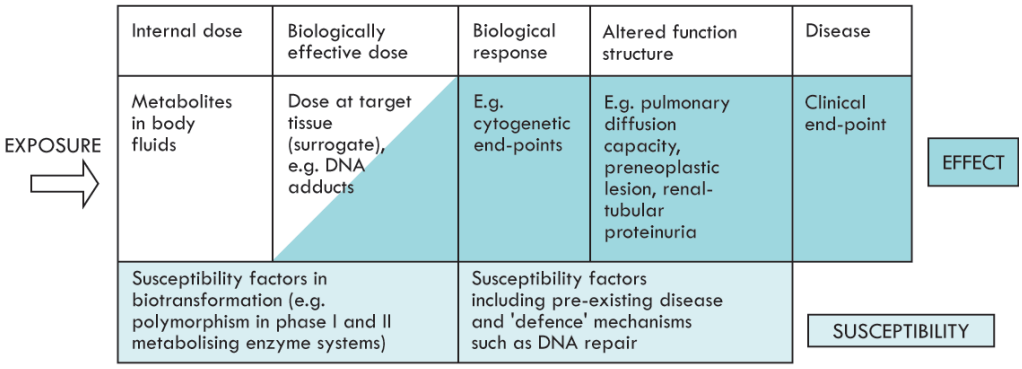
In much of the published work, biomarkers of effect have been used as possible indicators of the risk of ill-health and to construct dose–effect models in combination with indices of exposure. Thus a great deal of effort and debate has centred around the prognostic relevance of many effect biomarkers in defining increased risk of specific clinical disease. For example, there has been considerable debate about the relevance of screening relatively unselected populations using urinary markers of nephrotoxicity (even using such established tests such as urinary albumin), for the detection of individuals with significant risk of clinical renal disease; there is a need to validate the prognostic health significance of effect biomarkers by prospective studies in cohorts with a high risk of the clinical end-point(s) (USA/European Union, 1996).

There are a number of reasons for using biomarkers of effect as indicators of exposure assessment (Figure 2.5.1). For example:

- they require less invasive sampling than that needed for an exposure biomarker
- they may be able to provide information on dose to a target tissue that cannot be obtained from the measurement of the agent or a metabolite in a body fluid
- they give a combined picture of both biologically effective dose and the factors that determine expression of effect, such as genetic and acquired susceptibility
- they are suitable for use in large-scale epidemiological surveys in terms of cost, quality assurance of analysis and timing of sample (kinetics)
- there is a need to consider multiple or complex exposures which act additively or synergistically on a common pathway.

However, it is common for effect biomarkers to be used in combination with markers of exposure in order to maximise the degree of exposure information obtained.

Figure 2.5.1 Biomarkers of exposure effect and susceptibility*



* Adapted from National Research Council Committee on Biological Markers (1987); and Söderkvist & Axelson (1995)

METALS

Lead

The measurement of whole-blood lead is well established as a good index of recent exposure to inorganic lead; the half-life of lead in blood is approximately 32 days. The development of *in vivo* measurements for bone lead levels (Somervaille *et al.,* 1988; Watanabe *et al.,* 1994) allows the measurement of integrated, cumulative exposure; the half-life of lead in cortical bone is greater than ten years, but is shorter in the more metabolically active trabecular bone.

Several biomarkers of the effect of lead, mainly based on the interference of lead at several stages of the haem synthesis pathway, have been studied. Zinc protoporphyrin (ZPP) has been widely used in occupational and environmental studies. Significant increases in ZPP only occur in normal subjects at blood lead levels of above approximately 30 µg/dl. However, the time lag in increases in ZPP in newly exposed subjects, and ZPP elevation for a considerable time after cessation of exposure, in contrast to the decrease in blood lead, reflect the fact that ZPP is a

measure of the effective lead dose in bone marrow. The erythrocyte enzyme, *delta*-aminolevulinic acid dehydratase (δ-ALAD), is significantly inhibited at lower blood lead levels than those which cause alterations to erythrocyte ZPP levels. However, the instability of the enzyme, its inhibition by other chemicals such as mercury, carbon monoxide and inorganic arsenic, and methodological influences on the measurement have led to conflicting reports on its usefulness in low lead exposures. Critically, and in contrast to ZPP, the immediate inactivation of δ-ALAD following lead exposure and the ease of *in vitro* inhibition by lead indicate that blood δ-ALAD activity tends to reflect the concentration of lead in the blood compartment. The erythrocyte enzyme, pyrimidine-5-nucleotidase (5PN), is also significantly inhibited at blood lead levels as low as 10–15 µg/dl. Urinary measurements of aminolevulinic acid and coproporphyrin (mostly coproporphyrin III) have been used as indicators of altered haemopoiesis, but significant increases are only seen at blood lead levels of 40 µg/dl and above.

Both ZPP and δ-ALAD may have uses as biomarkers of susceptibility for lead exposure. Erythrocyte ZPP is elevated in subclinical iron deficiency, a not uncommon disorder in which haem synthesis is impaired and susceptible to the further toxic effects of lead. Thus, levels of ZPP that appear inappropriately high when compared with an individual's blood lead level may describe the combined effects of lead and iron deficiency. There has been debate about whether a relatively common variant of δ-ALAD (δ-ALAD-2 allele found in approximately 15% of Caucasians) is associated with susceptibility to higher blood lead levels in children and workers (Wetmur *et al.,* 1991). A recent report (Smith *et al.,* 1995) found that in subjects with low exposure (mean blood lead levels of 8 µg/dl), those with the δ-ALAD-2 allele did not have higher blood lead levels. However, they showed significant differences in the distribution and storage of lead between trabecular and cortical bone, suggesting an influence of the δ-ALAD-2 allele on the pharmacokinetic distribution of lead. There was also some evidence that the δ-ALAD-2 genotype may be associated with biochemical changes associated with lead nephrotoxicity.

Mercury

The measurement of urinary mercury is a useful exposure biomarker. Increases in urinary mercury levels after the administration of 2,3-dimercaptosuccinic acid (DMSA) have been suggested to reflect the level of mercury stored in the kidney (Roels *et al.,* 1991). Mercury selectively alters porphyrin metabolism in the kidney proximal tubule, leading to a characteristic pattern, determined by high-

performance liquid chromatography (HPLC), of increased excretion of the 4- and 5-carboxyporphyrins. Significant increases in excretion of three to four times have been noted in dentists with relatively low exposure to mercury (Woods *et al.,* 1993) and a recent report using a DMSA challenge suggests that urinary (copro) porphyrin levels may reflect the mercury burden in the kidney in those with low-level occupational and environmental exposure (Gonzalez-Ramirez *et al.,* 1995).

Cadmium

The increased excretion of low molecular weight proteins in cadmium exposure is considered an effect biomarker that indicates a very early adverse change in renal function. The measurement of urinary proteins, such as retinol-binding protein and *alpha*-1-microglobulin, which are stable in acidic urine, allows detection of very small changes in the fractional reabsorption of proteins in the renal proximal tubules. In large-scale epidemiological studies, significant increases in low molecular weight proteinuria have been noted, with creatinine levels of around 5μg/g or even lower (Buchet *et al.,* 1990). This may suggest that those who are more susceptible to renal tubular damage are being detected. Metallothionein is a low molecular weight metal-binding protein that is induced in many tissues by several metals including cadmium, zinc, copper and mercury. Urinary metallothionein has been suggested to be a measure of the kidney burden of cadmium but, in mixed low-level exposure may reflect the combined effect of metallothionein-inducing divalent metals.

PESTICIDES

Measurements of depressed plasma cholinesterase (ChE) and erythrocyte acetylcholinesterase (AcChE) activities are widely used in both the diagnosis of clinical organophosphorus pesticide (OP) poisoning and occupational OP exposure. For the OPs registered for use in the UK, ChE, which is less 'toxicologically relevant' than AcChE, is more sensitive to inhibition. ChE and AcChE have different half-lives in blood and undergo subsequent reactions of spontaneous reactivation and irreversible inactivation (ageing) at different rates. Wide interindividual variation in ChE activity has meant that serial measurements or 'baseline' levels in an individual are necessary in order to detect subclinical changes. The use of specific-activity ChE plasma measurements (activity/concentration) has provided both a means of interpreting single blood sample measurements and a more sensitive method of detecting OP absorption (Brock *et al.,* 1990).

The fact that approximately 80% of UK registered OPs are metabolised to one or more of six dialkyl phosphate and phosphorothioate metabolites has led to the development of a sensitive tool for the detection of low exposures to OPs (Nutley & Cocker, 1993). Gas chromatography with flame photometric detection can readily detect urinary alkyl phosphates at low nmol/mmol creatinine levels. Comparisons have shown that urinary alkyl phosphate measurements can readily detect exposures well below those detectable by plasma ChE measurements. However, the measurement of urinary alkyl phosphates cannot distinguish between absorption of active parent OP and preformed inactive metabolites, for instance from dietary sources. The half-life of urinary alkyl phosphates after a single exposure is approximately five to seven hours.

Whilst plasma ChE is considered to be a protective 'sink' for absorbed OPs, protecting neural target sites, there is continued debate about the role of 'paraoxonase' and its genetic variants in determining an individual's susceptibility to various OPs. Paraoxonase can hydrolyse a range of OPs, including chlorpyrifos, diazinon and pirimiphos-methyl, to inactive metabolites. Animal studies have shown that the level of activity of serum paraoxonase towards an OP can influence the toxicity (Costa & Manzo, 1995; Li *et al.,* 1995). However, epidemiological studies in humans to determine the relevance of the level of the enzyme activity and its polymorphism to susceptibility to OP toxicity are still lacking. The ability to measure routinely, in those with low-level exposure, urine alkyl phosphate metabolites resulting from OP hydrolysis may help to resolve this question.

SOLVENTS

The vast majority of published work on biomarkers of effect has concentrated on their use as early indicators of potential health effects, whether to the nervous, liver, reproductive and kidney systems, or genotoxicity (Baker, 1994).

Some recent studies have explored the influence of genetic polymorphism or acquired changes in enzyme systems metabolising chemicals such as dichloromethane, trichloroethylene and toluene (Kawamoto *et al.,* 1993; Hallier *et al.,* 1994). Such studies on occupational exposure have begun to highlight the effect of these changes on routine biomarkers of exposure (carboxyhaemoglobin for dichloromethane, urinary hippuric acid for toluene, urinary S-methylcysteine for methyl bromide), and also the racial differences in the prevalence of some of these polymorphisms (see Table 2.5.1).

Table 2.5.1 Metabolic enzyme systems that may affect biotransformation of xenobiotics*

	Toxicological substrate	Comments, assays
Cytochrome P450		
1A1	PAH	Inducible; polymorphic with regard to inducibility; EROD assay or RT-PCR levels of mRNA 1A1 in lymphocytes
1A2	Arylamine, nitrosamines	Inducible, assay caffeine clearance
2A6	Nitrosamines, aflatoxin B	Inactive variant allele frequency of 2% in Caucasians
2D6	Nitrosamines, procarcinogen tobacco	'Debrisoquine metabolism phenotype' genotyping: 5% 'poor metabolisers' in Europeans, 1% in Japanese
2E1	Alkanes, 1,3-butadiene, ethanol, benzene, halogenated hydrocarbons	Ethanol-inducible, polymorphic, high interindividual variability, produces oxy radicals, immunochemical assay on lymphocytes or chlorzoxazone metabolism
3A4	Aflatoxin B_1, broad specificity	Inducible, large quantity in liver, assay 6-ß-hydroxycortisol excretion
Paraoxonase	Hydrolyses OP and carbamate pesticides to inactive metabolites	Serum/liver enzyme, polymorphic for some OPs (50% low activity, 10% high activity); not for other OP substrates (e.g. chlorpyrifos) where activity reflects concentration
Phase II metabolism		
GST mu	Epoxides of styrene	50% of Europeans have null allele; immunochemical assay in lymphocytes
GST theta	Dichloromethane, methyl bromide, ethylene oxide	25–30% have null GST1 gene, low metabolism genotype assay or *in vitro* blood metabolism of methylbromide
n-Acetyltransferase II (NAT-2)	Aromatic amines	5 Genotypes; 3 activity levels - low, intermediate and high acetylators, genotype or caffeine metabolic phenotyping

EROD, ethoxyresorufin-*o*-deethylase; RT-PCR, reverse transcriptase polymerase chain reaction; OP, organophosphorus pesticide;

* From *Daly et al.,* 1993

MISCELLANEOUS BIOMARKERS OF EFFECT

Insults to the lung are common in environmental and occupational exposure, whether by reactive gases (nitrogen oxides/ozone) or particulates. Physiological lung function testing remains a prime method of detecting their effect. Recent work (Bernard & Lauwerys, 1995) suggests that the measurement of the protein CC16 in serum may reflect toxic effects on Clara cells localised in the terminal bronchioles of the lung. Clara cells act as progenitor cells for both themselves and ciliated cells following injury to the bronchiolar epithelium; depletion of Clara cells is therefore likely to compromise repair mechanisms in this area. These cells also contain most of the lung cytochrome P450 activity and are involved in the activation/transformation of many cytotoxic and carcinogenic chemicals. Many chemical exposures, including exposures to environmental pollutants and smoking, have been shown to damage Clara cells (Richards *et al.,* 1990). Bernard and Lauwerys postulate that CC16 protein, a small protein found in high concentrations in respiratory tract fluid, diffuses into the bloodstream. They have shown significant decreases in serum CC16 protein in smokers and subjects exposed to lung toxicants (silica, welding fume). Effects appear to be additive (Bernard & Lauwerys, 1995).

It has been suggested that cellular response markers of acute inflammation, detected in nasal lavages, could be used to monitor the effects of low-level ozone exposure (Devlin, 1993). The measurement of exhaled endogenous nitric oxide may have uses in detecting inflammatory responses in the upper and lower airways (Kharitonov *et al.,* 1995; Schedin *et al.,* 1995).

Subtle alterations in immunoregulation (Kolopp-Sarda *et al.,* 1995; Tanigawa *et al.,* 1995) can now be easily detected from changes in the surface antigens on circulating blood cells, using the technique of flow cytometry. Changes in the type and concentration of immunocompetent cells in blood may represent homeostatic mechanisms in the immune system of healthy individuals in response to toxic insults, or very early markers of a disease state.

2.5.3 BIOMARKERS OF SUSCEPTIBILITY

Table 2.5.1 shows some of the metabolic enzyme systems which, either by genetic or acquired changes, may lead to individual differences in the biotransformation of commonly encountered chemicals. Such differences may alter the biologically effective dose. In this complex area, thought must be given to: (*a*) whether measurements of the levels of expressed enzyme in a surrogate tissue (e.g. lymphocytes) reflect levels in target organ or tissue; and (*b*) the fact that despite the rapid advances in discovering genotypic differences, metabolic polymorphism (if present) is central to the influence on biotransformation.

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2.6 BIOMARKERS IN WILDLIFE: POSSIBLE SURROGATES FOR HUMAN EXPOSURE

D Osborn

Institute of Terrestrial Ecology, Monks Wood, Huntingdon

2.6.1 INTRODUCTION

Using biomarkers in wildlife (or domesticated animals) as surrogates for human exposure is not a new concept. When coal-miners took canaries down mines, for example, they were using the response of the canary as a biomarker of both exposure and effect. This paper considers some of the factors that might need to be considered in developing the use of wildlife biomarkers in this way.

2.6.2 WILDLIFE SURROGATES

The natural tendency in considering wildlife surrogates is to think first and foremost of mammals. However, birds have many advantages and, depending on the aims of a study, the use of other vertebrates and invertebrates might also be considered. In certain instances (e.g. in studying air pollution) plant effect biomarkers could also be used as surrogates for human exposure.

Sampling strategies are a key issue in any work on surrogates. It would not generally be a practical proposition to place surrogates alongside exposed people, although the development of very small biologically-based sensors is not beyond the bounds of possibility. Therefore, in order to achieve sufficient similarity between human and wildlife exposure patterns, careful consideration should be given to the proximity of wildlife surrogates to exposed humans. This would determine whether organisms living within dwellings, in gardens or in more public places offered the best opportunity for study.

In any study using surrogate organisms it is particularly important to be sure that the use of the surrogate addresses the issues concerned. For example, if there is a need for quantitative information, rather than simple qualitative information, then a reasonably thorough understanding of dose–related links between human and wildlife exposure is required if the use of the surrogate is to be successful.

It is also necessary to be sure that direct methods of chemical analysis are not the most suitable way of obtaining an adequate estimate of human exposure for regulatory or health purposes. Equally, sampling people directly should always be considered and the surrogate approach used only if the more direct approach seems unjustified, for example, on practical, moral or ethical grounds.

Surrogates might well prove useful in monitoring impacts of accidents or in studies of general environmental contamination (e.g. in large cities); the approach would, however, be very different in these two cases: in one chemical release is sudden and in some ways localised, and in the other contamination is more diffuse (hotspots in the general background might exist that could be identified using wildlife surrogates).

Although wild animals have many aspects of biochemistry and physiology in common with humans, they have a number of inherent characteristics that need to be taken in account, such as the seasonal nature of their physiology and physiological features associated with their evolution (e.g. birds have special structures associated with flight and egg-laying). These commonalities and differences need to be understood when selecting surrogate biomarkers. In the first instance it might be best to restrict work on surrogates to the commonly shared systems, since this would give a greater chance of ensuring that the surrogate response was one that was relevant to humans.

There is a growing body of evidence that wildlife biomarkers are useful indicators of the impact of human activity. Biomarkers that indicate an organism is suffering

some undefined stress and biomarkers specific for exposure to particular chemicals are either being developed *de novo* or applied to wildlife work from their origins in human studies. Recent work in the terrestrial environment has shown that the impact zones of industrial/commercial accidents (Svendsen *et al.,* 1996) and industrial activity can be identified (Weeks & Williams, 1994). Such results could be used to help focus health studies on humans living near such sites by identifying the areas in which work should be concentrated.

To use a surrogate approach successfully it will be necessary to establish that the wildlife species used experience similar (or greater) exposure than humans and that both have similar susceptibility. Very little is known about interindividual or interspecies variation in susceptibility in terrestrial species.

2.6.3 POSSIBLE USES OF SURROGATE BIOMARKERS

If exposure and sensitivity of a proposed surrogate were higher than that in humans then it might be used:

- to define worst case conditions
- to provide an 'early warning' or indication of possible effects on humans
- to provide an economically effective way of ranking human health questions by indicating the types of responses
- to assess whether exposure was from chemicals passing through food-chains or chemicals being inhaled.

In order to advance the use of biomarkers in wildlife as surrogates for human exposure the following approach might be used:

- establish baseline conditions for a suite of generic and chemical-specific biomarkers; this would need to consider dose–response relationships, exposure, sensitivity and susceptibility issues

- conduct a stratified sampling regime using known contamination gradients to validate the response of the biomarkers in the field
- test the predictive or indicative capability of the surrogate approach with a biomarker common to human and wildlife studies (preferably one that is common to both terrestrial and aquatic systems and relatively inexpensive to use, e.g. lead or aminolevulinic acid dehydratase levels in blood)
- examine the possibility that a wildlife response biomarker could be used to obtain quantitative information about human exposure.

The approach might best be tested in an urban area where wildlife and people are in close proximity. The use of plants as well as animals should be considered as should the possibility that the surrogates might be specially brought into an area in an equivalent of the 'canary's cage' to increase the chances of controlling confounding variables.

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3 Assessment of exposure to pollutants *via* air, water, food and multiple pathways: The application of biomarkers

3.1 ROUTES OF EXPOSURE

3.1.1 AIR

D Gompertz and AJ McMichael

A range of human activities result in the release into the air of a variety of toxic materials. This is not a new phenomenon, for example, the burning of coal in domestic and industrial activities was associated with the severe episodes of atmospheric pollution seen in the UK earlier in the twentieth century. The subsequent change to a different pattern of fossil fuel use has been associated with a change in the pattern of pollution, but one that is still associated with human respiratory disease (IEH, 1994). The increase in vehicle emissions is increasingly superimposed on the pattern of pollution from the direct combustion of fossil fuels for power generation and heating; together they are responsible for the levels of inorganic gases (nitrogen dioxides (NO_x), sulphur dioxide (SO_2) and, indirectly, ozone (O_3)) and fine particulates (PM_{10} fraction) currently observed (MAAPE, 1991, 1992, 1993; Committee on the Medical Effects of Air Pollutants, 1995). Inorganic metal contaminants including lead and now platinum are produced from petrol fuelled transport.

Industrial activities have long resulted in the emission of toxic metals into the air. Air pollution around smelters has occurred since historical times and is still happening. More recently the incineration of domestic waste has resulted in emissions of various pollutants, including toxic metals, fine particulates and polychlorinated polycyclic hydrocarbons (Royal Commission on Environmental Pollution, 1993).

Toxic materials accumulate in indoor air from the outgassing of pollutants from building and other materials, and from the generation of combustion gases (carbon monoxide (CO) and NO_x) particularly from gas cooking (IEH, 1996). Environmental tobacco smoke is a further important indoor air pollutant.

Although human exposure to these airborne pollutants occurs by inhalation, this need not be the major pathway, and absorption can occur following deposition on food and crops, directly or indirectly, or into water or on surfaces.

Exposure to airborne pollutants may be uniform over a large geographical area or can be relatively localised. Both SO_2 and NO_x can be transported over large distances, but local concentrations of NO_x and particles are affected by proximity to roads and other sources. Ozone, a secondary pollutant generated by the interaction of NO_x and volatile organic compounds (VOCs), is often generated at some distance from the source of its precursors.

Personal exposures are affected by the local pattern of exposure as well as by individual activities, such as the time spent indoors, in cars and outside. Establishing the health effects of various activities and exposures requires information about individual levels of exposure, and biomarkers can be useful for this purpose. The measurement of blood lead concentrations in various population groups over the last two decades has shown how a well established biomarker of individual uptake can be used to monitor the effectiveness of efforts to control airborne exposure to a toxic hazard, in this case lead in petrol.

Priorities for the development of appropriate biomonitoring methods for airborne pollutants relate to the current pollutants of international concern. There is a great need for methods that give a measure of individual exposure to PM_{10} and other fine particles and, related to this, for biomarkers to assess exposure to inorganic gases. Methods for assessing recent exposure to benzene and polycylic aromatic hydrocarbons (PAHs) are available, but some measure of long-term past exposure would aid epidemiological studies; current DNA and protein adduct techniques essentially reflect exposure over months, not years. It is possible that newer molecular biology techniques, such as the use of (putatively highly specific) mutational spectra, may indicate the patterns of past exposure long after the toxic species have left the body.

As biomarkers are developed to reflect respiratory uptake of airborne materials, studies are also required of individual and environmental factors that modify the relationship between the inspired dose and the level of the biomarker in the body fluid. Genetic polymorphisms may well affect the measurement of a metabolite biomarker (but this will be so whether the parent substance is taken in by inhalation, by ingestion or through the skin). Co-existing respiratory impairment, the use of inhaled medication and variations in exercise need to be considered as modifiers, as also do age and smoking habits.

Biomarkers are well developed for a range of occupational chemical exposures, and some of these, mainly the biomarkers for toxic metal exposure, are suitable for environmental exposure assessment. Investigation and control of a number of important airborne environmental contaminants (PM_{10}, PAHs, polychlorinated polycyclic compounds, VOCs and inorganic gases) would be aided if effective low-cost biomarkers were available.

3.1.2 WATER

P Calow and C Walker

Drinking-water and surface waters (i.e. lakes, rivers and seas) present rather different problems with respect to contamination and human risk assessment and are therefore discussed separately.

DRINKING-WATER

Drinking-water usually contains very low levels of contaminants, some of the most important of which arise as a consequence of treatment and delivery (e.g. disinfection by-products and lead from old pipes). The major concern relates to ingestion of water; a minor concern is exposure *via* the skin, for example, when taking baths and showers.

Disinfection by-products in drinking-water pose a particularly difficult problem. The chlorination process can generate many trace contaminants (e.g. chlorinated phenols) as a result of interaction between the dissolved gas and organic compounds. Measuring human exposure to such a mixture of compounds would require a wide range of techniques. At the moment there is no collection of biomarker assays to meet this requirement, although certain individual contaminants could be measured by existing analytical methods.

There have been occasional short-term incidents concerning contamination of drinking-water. Two such examples in the UK are the River Dee incident involving phenols (Jarvis *et al.*, 1985) and the Camelford incident involving aluminium sulphate (Rowland *et al.*, 1990). Exposure to phenol can be established

by analysis of urine, exposure to aluminium by analysis of serum and urine. It would be difficult to produce comprehensive guidelines for occasional unpredictable episodes such as these. Nonetheless, as a minimum, samples of blood and urine should be taken at the time of the incident, the date and time recorded for each sample and the samples stored at deep-freeze temperature for future analysis. Such samples would have greatly facilitated the subsequent investigation of the Camelford incident.

SURFACE WATERS

Surface waters vary enormously with regard to the nature and quantity of pollutants they contain. The most important sources of human exposure associated with aquatic pollution are the consumption of contaminated fish, shellfish, and products from marine mammals. Exposure to pollutants may also occur from recreational activities such as bathing and diving in lakes, rivers and seas.

The range of pollutants found in lakes, rivers and seas is disturbingly large. The metals cadmium, lead, copper and mercury and zinc, organomercury, organolead and organotin compounds, PAHs, organochlorine insecticides, polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins (dioxins) are pertinent examples. There is a problem with local 'hot spots' at or near places of release, for example, sewage outfalls, industrial waste outfalls, oil terminals and wrecked ships; also there are places where pollutants are concentrated, for example river estuaries where contaminated particles are deposited. Furthermore, the question of biomagnification of persistent pollutants must be considered; for example, PCBs and dioxins in marine and freshwater food-chains can give rise to relatively high levels of contamination in predatory fish, mammals and birds.

The transfer of pollutants in aquatic systems is complex, and further studies would benefit from the availability of more and better biomarkers. Individual organisms are exposed to pollutants in water, in sediment, and in their food. Currently there is concern about the substantial levels of certain pollutants, such as metals and organic compounds, in sediments, and the extent to which these are available to bottom-dwelling organisms. There is also concern about the concentration of pollutants in sewage sludge, since sludges are often spread on agricultural land as organic fertiliser and soil conditioner, thereby providing a route for these pollutants into the human food-chain.

An ecological approach is necessary for a better understanding of aquatic pollution. Apart from the diversity of chemicals and their transformation products, consideration must be given to the biological diversity of aquatic ecosystems and to taxonomic, genetic and environmental variability (Forbes, 1996). The successful use of biomarker assays depends on an understanding of such variability. Levels of pollutants in surface waters are sometimes high enough to have lethal or sublethal effects in individual organisms, leading to population declines, for example, where an oil tanker is wrecked, or where tributyl tin (in antifouling paint) is released into shallow waters. The effective use of biomarkers can give a measure of both exposure and toxic effect in such situations.

This approach is not without relevance to human risk assessment. In the most immediate sense it can give warning of the contamination of fish and shellfish intended for human consumption. The detection of mercury and organochlorine insecticides in marine organisms has provided evidence of undesirably high human exposure to these chemicals. A biomarker of exposure responsive to a wide range of pollutants can give a cost-effective indication of chemical contamination of fish/shellfish. Where a biomarker assay indicates exposure, this may then be followed by chemical analyses to identify the pollutants concerned.

Table 3.1.1 gives examples of biomarker assays that have been used in aquatic organisms (Depledge, 1993; Moore *et al*., 1993). They are arranged approximately in order of decreasing specificity. Thus *delta*-aminolevulinic acid dehydratase (δ ALAD) inhibition in fish is specific for lead, and the induction of imposex in certain molluscs appears to be specific for tributyl tin. Less specific biomarkers are indicative of exposure to groups of chemicals. Metallothionein is induced by a range of metals, ethoxyresorufin-*o*-deethylase (EROD) by planar organic compounds, DNA adducts by a range of organic molecules. Some of the techniques listed, for example, brain acetyl cholinesterase inhibition, are biomarkers of toxic effect as well as exposure. Furthermore, some biomarkers of toxic effect also give integrative measures for groups of compounds (e.g. the aryl hydrocarbon receptor; see p52 and Safe, 1994). There can be considerable advantages in using appropriate combinations of biomarkers which are specific and nonspecific, and measuring responses at different levels of organisation (e.g. molecular and cellular).

Table 3.1.1 Biomarkers of exposure to contaminants in aquatic organisms (in descending order of specificity)

Biomarker	Organism	Contaminants	Comments
Tissue residues	Any organism	Many	Highly specific, but mainly applies to bioaccumulated or persistent substances
Fluorescent bile metabolites	Fish	PAHs	A particular example of diagnostic residue analysis
ALAD inhibition	Fish	Lead	Very specific biomarker of exposure
Induction of imposex/intersex	Female neogastropod molluscs/female littorinid molluscs	TBT	Possibly uniquely diagnostic of exposure to TBT accompanied by testosterone induction
AChE inhibition	Fish; crustaceans; bivalve molluscs	Organophosphorus insecticides; carbamates; some algal toxins	A fairly specific biomarker of exposure
Metallothionein induction	Fish and vertebrates	Certain metals, including zinc, copper, cadmium and mercury	
EROD or P4501A1 induction	Fish	Planar organic compounds (e.g. some PCBs, PAHs, dioxins)	Sensitive indicator of exposure. May also predict pathology
Bulky DNA adduct formation	Fish; bivalve molluscs	PAHs, some other organic compounds (e.g. nitro compounds), and some pesticides (e.g.triazines)	Estimates genotoxic potential
Neoplastic liver histopathology	Fish	PAHs, some other organic compounds and some pesticides	Pathological change associated with carcinogenesis

Table 3.1.1 (cont.)

Biomarker	Organism	Contaminants	Comments
Activation of (*ras*) oncogenes or damage to tumour suppressor genes		PAHs, some other organic compounds, and some pesticides	Predictor of carcinogenic effects
Vitellogenin induction	Male fish	Oestrogens and oestrogen mimics	Oestrogen mimics include a very diverse range of substances
Sex hormone induction	Fish and some invertebrates	Endocrine disrupting substances	E.g. elevated testosterone in females
Lysosomal accumulation	Any	Many xenobiotic compounds	
Scope for growth	Invertebrates	Many xenobiotic compounds	

ALAD, aminolevulinic acid dehydratase; AChE, acetylcholinesterase; EROD, ethoxyresorufin-*o*-deethylase P4501A1, Cytochrome P4501A1; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; TBT, tributyl tin

3.1.3 DIETARY EXPOSURE TO ENVIRONMENTAL POLLUTANTS

S Bingham and DEG Shuker

Contamination of food supplies is monitored using various surveillance procedures. In the UK these consist partly of analyses of 'market basket' samples, for example the Total Diet Study (TDS); levels are then compared with acceptable levels, which should assess the likelihood of individual excess exposure (Ministry of Agriculture, Fisheries and Food, 1994). However, for analytical epidemiology, validation of surveillance techniques and assessment of particular groups it is necessary to assess the actual intakes of individuals. Biomarkers of exposure are potentially a useful alternative to the well established methods for measurement of food intake.

Measurement of many of the biomarkers of potential use for assessing human exposure to trace chemicals from ingestion of food has developed through the monitoring of occupational exposures. One of the key questions is whether these techniques are sufficiently sensitive to detect the much lower exposures associated with dietary intake, for example, to pesticides, PAHs and metals. Other questions have arisen following recent findings of potential dietary hazards, for example heterocyclic aromatic amines (HAA) present in cooked meat and fish, where the chemicals are present as a consequence of cooking by the consumer (Eisenbrand & Tang, 1993; Layton *et al*., 1995).

For a given dose, uptake of environmental pollutants from food can be strongly influenced by other dietary constituents, for example, chelators which affect absorption (such as phytic acid), competition at absorptive sites (such as between trace elements) and bacterial metabolism by the intestinal flora. There may be several routes of excretion (including sweat, breast milk, faeces), which could be useful for sampling, in addition to the most widely used media, blood and urine. Body composition changes may also alter plasma levels of fat-soluble contaminants. Since there are some 5000 different food items available for choice in human societies, most diets are subject to wide seasonal, geographical and social variations. To be useful, the chosen biomarker must be able to show a predictable relationship between the dose ingested and the biomarker measure at comparatively low and varying dietary levels.

Further considerations concerning the potential use of biomarkers include the accessibility of suitable biological samples, which may range, for example, from tissue biopsy or 24-hour urine or faecal samples to a single blood specimen, spot urine, hair samples, or toenail cuttings. Biomarkers that reflect habitual long-term exposure may be required for studies of cancer outcome, whereas the power of the biomarker to reflect recent short-term ingestion may be important for pesticide and metal poisonings. Analysis of trace metals has been long established and there are good quality control procedures in place, but this is not yet the case for newer techniques including measurement of DNA and protein adducts.

The needs and current status of possible biomarkers for selected pollutants in food should be considered with the above criteria in mind. In addition to contaminants such as pesticides, metals, HAAs, and PAHs, biomarkers for nitrate may also be considered. A high level of nitrate in water is a cause of methaemoglobinaemia in infants. There are also concerns about the role of nitrate/nitrite in the aetiology of gastrointestinal tract cancers. Classification of nitrate as a food contaminant does, however, have to be viewed in the light of the recent elucidation of substantial endogenous synthesis of nitrate in the human body, as well as the influence of gaseous nitrogen oxides from combustion sources on the total body burden of nitrate.

3.1.4 MULTIPLE PATHWAYS

P Toniolo and NJ King

Compared with traditional exposure assessment methods, such as questionnaires, employment records and ambient monitoring, biomarkers offer the advantage of adding biological relevance to epidemiological studies and improving the mechanistic understanding of disease causation (Hulka *et al.*, 1990). For example, measurements of DNA adducts in target cells provide insights into the effects of exposure at the cellular level which could not be achieved otherwise. The use of biomarkers also adds a degree of flexibility that traditional methods could not offer. By using different or related biomarkers in sequence or in combination, or by applying the same biomarker to different target tissues or biological media, the possibility of investigating the biological consequences of exposure in humans is greatly expanded. Moreover, exposure–effect relationships can be examined

simultaneously from many different angles. This potential has not been fully appreciated nor exploited as yet, possibly because the number of available, and dependable, biomarkers of exposure is still too limited.

In the future, the ability of biomarkers to be used in sequence and/or in combination will allow investigators to address, in much greater detail than is possible today, the relative role of exposures from multiple pathways, from different routes of absorption and the significance of metabolism. Such applications are still at an early stage of development.

BIOMARKERS TO HELP DISCRIMINATE BETWEEN DIFFERENT SOURCES AND ROUTES OF EXPOSURE

The availability of biomarkers that are specifically related to single sources or routes of exposure would be of considerable value. There are few that have been used in practice. The best example is the measurement of stable isotopes of lead in blood and in deciduous teeth (Delves & Campbell, 1993; Alexander *et al*., 1993; Gulson & Wilson, 1994). The application depends on the requirement that different sources of the metal are isotopically distinguishable. The ratio of different isotopes in target tissue will indicate the likelihood of a specific source. For example, it has been shown that leaded petrol (gasoline) has a lower ^{206}Pb : ^{207}Pb ratio than lead from drinking-water pipes (see p16). The major limitations have to do with the accessibility of test tissues (e.g. teeth), the lack of reliable data on specific isotopic composition of the source (e.g. in food, drinking-water, air, paint, dust and soil), and the discriminating ability of specific isotope ratios in target tissues. Nevertheless, the approach is promising and has the advantage that it could potentially be extended to other compounds.

Speciation of urinary arsenic and its metabolites has been used to differentiate between occupational exposure to inorganic arsenic and dietary exposure to less toxic organic arsenic species present in seafood (Lauwerys & Hoet, 1993). Biomarkers can also be used to distinguish between endogenous and exogenous nitrate (Perciballi *et al*., 1989) but other examples are few and much more work is needed.

Measurements of specific compounds in different tissues, such as in blood, urine, breath, saliva and lymphocytes, have been proposed and may have some discriminating value, although the relevance of different measurements in different tissues is often unclear.

BIOMARKERS TO PROVIDE INFORMATION ABOUT HISTORY OF EXPOSURE

Biomarkers could be useful in assessing past exposure to specific compounds more precisely than traditional exposure assessment methods. Typically, measurements of selenium, lead and other metals in teeth or bone have been used for precisely this purpose. In occupational studies, markers of internal dose have been used in temporal sequence to gather information on fluctuations in ambient exposure over time. For example, excretion of chromium in urine, which can be easily measured in spot urine samples obtained at specified intervals, could provide a fair picture of short-term total chromium burden, whereas concentrations of chromium in peripheral lymphocytes or in erythrocytes will provide an estimate of cumulated exposure to hexavalent chromium. Highly liposoluble compounds, such as certain organochlorine compounds, serve as another model. For example, DDT concentrations in blood or fat tissue will provide an estimate of exposures during the previous several months, whereas measurement of its persistent metabolite DDE will reflect exposures dating back many years, if not decades. The major limitation to a more widespread use of biomarkers as indicators of past exposures is that few are indicators of medium- or long-term (i.e. years of) exposure. Many of the more volatile pollutants (solvents, VOCs, inorganic gases) have half-lives in tissues that are too short (hours) to allow them to be used as biomarkers of exposure. However, some are metabolised to substances with longer half-lives (e.g. trichloroethylene) and these metabolites can be used to monitor recent exposures. Protein and DNA adducts are also limited in how much information that they can give about longer-term past exposures. The measurement of adducts of histone proteins in bone marrow cells may, however, provide the basis for a longer-term measure of exposure to benzene.

COMMENTS

While potentially providing a more accurate index of individual exposure, the use of a new biomarker may involve more complex issues than the more traditional exposure assessment methods used in epidemiological studies. An understanding is needed of how uptake and metabolism vary with time and between individuals, and how this can affect measurements, before biomarkers can be used effectively to study exposure through either single or multiple pathways.

When detailed occupational exposure records are available, estimates of individual exposure to a given chemical can be satisfactorily constructed for many epidemiological purposes. These exposure estimates can be integrated over time to give long-term, albeit imprecise, indices of exposure. In spite of this imprecision, epidemiological studies relating exposure at the group level with outcome are both feasible and interpretable. But if a highly sensitive and specific biomarker of long-term exposure to that particular compound was available, much more accurate individual exposure information could be produced. The value of the information may, however, be crucially dependent on an extensive knowledge of interindividual variability in the kinetics of uptake and metabolism, as well as on differences in absorption route. The variability in measurements within and between individuals will not, though, be a function solely of external exposure; it will also be influenced by a number of potential confounders, such as diet, drugs, or competing chemicals, which may be capable of modifying the exposure index (the biomarker). Thus before some newer biomarkers of exposure can be used effectively, a substantial amount of preliminary work must be performed to document the sources and extent of these variabilities.

The use of biomarker measurements in wildlife or pets as surrogates for estimating human exposure in certain situations has been suggested (see also section 2.6), although they have seldom been utilised. Wildlife and pets could also be used to develop and validate biomarkers of exposure, including the preliminary assessment of the variability issues addressed above. Advantages include the similarity between the basic biochemistry of animals and humans, and the fact that animals and humans often share similar ambient exposures. In exposure studies, problems may arise from differences in exposure patterns between humans and animals. For example, pets may be much more heavily exposed to contaminated soil through skin contact and ingestion. Ethical and legal considerations may also limit their use in some countries. However, these problems and differences would be less important for developmental studies in which the relevant issue would be, for example, the preliminary assessment of variability parameters.

3.2 BIOMARKERS

3.2.1 REQUIREMENTS

Table 3.2.1 summarises some specific situations where improved methods to assess exposure to particular pollutants are required; in all these situations the use of biomarkers has potential advantages over other measures of exposure.

The potential use of biomarkers varies greatly between the different categories of pollutants. For example, the measurement of blood lead has been used to investigate the role of exposure in the development of children's intelligence (McMichael, 1995), to establish sources of exposure (e.g. vehicle emissions to air, paint, water) and to assess the effects of remedial action. Cadmium measurements have been used in the investigation of population exposures and health effects in contaminated industrial areas (Stæssen *et al*., 1994). PAH adducts have been investigated to establish the relative levels of environmental and occupational exposure to PAHs in urban and rural areas with differing levels of air pollution (Perera *et al.*, 1992).

The range of uses to which biomarkers have been applied has depended on the availability of the biomarker, the ease of use of the analytical technique (sensitivity and throughput) and the awareness of the toxicological problem. For example, the measurement of benzene biomarkers has moved from the workplace to the garage forecourt and the home, which has required a change in the biomarker used, since the need for increased sensitivity made the measurement of urinary phenols inadequate.

Table 3.2.1 Important requirements in relation to exposure assessment

Exposure	Application	Scale of analysis	Comment
Toxic metals			
Lead, cadmium, arsenic, mercury	Population surveillance for environmental and mixed pathway exposure; epidemiological studies of health effects in the general population and vulnerable subgroups	Large-scale (1000s) and local surveys (100s)	Methods and quality assurance techniques are well established
Chromium, nickel (manganese)	Local and specialised studies (e.g. near incinerators or smelters)	Local surveys (e.g. 100s)	Special attention to contamination problems required during analysis
Aluminium	Epidemiology, surveillance (routine and emergency)	Large-scale (1000s) and local surveys 100s	Contamination a major problem during analysis
Volatile organic compounds and aliphatic solvents			
Volatile organic compounds (VOCs) in general	Pilot studies, surveillance, environmental studies (e.g. indoor air), epidemiology	Small local (10s–100s) and large surveillance exercises (100s–1000s)	Improvement of techniques required, especially in quality assurance procedures
Butadiene (adducts)	Pilot and transitional studies required before surveillance and population studies are possible	Pilot and transitional surveys (10s–100s)	Adduct methods require validation
Aromatic compounds			
Benzene	Population surveillance, health effect studies	Surveillance (100s–1000s), adduct studies (10s-50s)	Suitable metabolite biomarkers exist, but adduct methods are not yet developed

Table 3.2.1 (cont.)

Exposure	Application	Scale of analysis	Comment
Toluene, xylenes styrene	Indoors as part of general VOC exposure studies, outdoors associated with gasoline exposure	10s–100s	Methodology requirements as for VOCs in general
Polycylic aromatic hydrocarbons (PAHs)	Biomarkers for vehicle exhaust exposure (fine particulates, PM_{10} fraction) and incinerators, short-term episodes and case–control and surveillance exercises	100s–1000s	Studies needed in several areas: (1) usefulness of marker PAHs (e.g. 1-hydroxypyrene) as a general surrogate for total PAH exposure; (2) quantitation of DNA-protein adducts; (3) effect markers (mutations - spectra)
Chlorinated organic compounds			
Aliphatic chlorinated solvents (VOCs)	General population studies including indoor air exposure; local studies associated with specific sources (e.g. dry-cleaning and chlorinated water)	10s–100s	Studies needed of exposure to chlorinated water in the home (showering) and outside (swimming pools)
Lindane, dieldrin	Surveillance and studies of small populations after exposure	10s–100s	To study domestic pesticide usage
Dioxins, furans, polychlorinated biphenyls	Short-term exposure incidents, general population surveillance, surveillance around specific sources (incinerators), health effects investigation	As large as possible (current methods have low throughput)	Screening methods needed as current analytical technology is too expensive and time-consuming

Table 3.2.1 (cont.)

Exposure	Application	Scale of analysis	Comment
Other environmental hazards			
Environmental tobacco smoke (ETS)	Health effect studies, study of ETS exposure as confounder	Large-scale (100s–1000s)	Measures of retrospective exposure required, transitional DNA adduct studies needed to distinguish PAH exposure from ETS and from other sources retrospectively
Nitrogen oxides (NO_x)	Assessment of short-term exposure episodes and integrated longer exposures for chronic health effect epidemiology	Personal exposure assessment for large numbers of individuals (100s–1000s)	Studies of exposure to both indoor and outdoor NO_x and the related health effects need a convenient exposure marker – either a personal sampler or a biomarker would be a useful epidemiological tool; a biomarker that integrates exposure would be helpful
Fine particulates (PM_{10} fraction)	Monitoring population exposure including routine surveillance, acute pollution episodes, checking control measures, epidemiological studies, case–control and large-scale studies	From medium (10s–50s) to large (1000s)	Markers based on specific components of PM_{10} exposure required urgently; non-invasive techniques for recovery of particles (e.g.induced sputum) would be useful

3.2.2 AVAILABILITY

The biomarkers that are available for different classes of pollutants (toxic metals, VOCs, chlorinated organic compounds, other organic compounds, inorganic gases, particulates) vary from class to class. As biomonitoring techniques increase in their availability, sensitivity, throughput, accuracy and precision, a number of new questions can be addressed. These include investigation of dose–response relationships for various clinical end-points, the relative importance of various sources of pollution, and the need for and effectiveness of controls. In Table 3.2.2 a list of currently available biomarkers is presented, indicating their state of development, relative cost and ease of analysis. The model of lead biomonitoring shows that the application of biomarkers to the various problems of exposure and disease causation increases as techniques become more readily available. This is also reflected in recent population studies of cadmium exposure. The use of ^{32}P postlabelling techniques to investigate PAH exposure around a polluting industrial site shows that the early use of monitoring techniques can give valuable insights, even in the absence of information about measurement and effect relationships.

TOXIC METALS

The use of biomarkers and the associated analytical techniques for measuring metals in body fluids are well established and validated (Lauwerys & Hoet, 1993) and many have sufficient sensitivity to determine normal levels in nominally unexposed populations. Both certified reference materials and national and international quality assurance schemes are available for the major metals of environmental interest. Furthermore, the factors affecting exposure and uptake and their relationship to the measured biomarker are largely understood.

Recent developments in isotopic techniques (see p12), such as inductively coupled plasma mass spectrometry (ICP-MS), have allowed differentiation between sources of lead pollution in various population studies. However, this technique is considerably more expensive than the routine measurement of blood lead concentrations. The greater use of assays that determine ratios of lead isotopes deserves serious consideration. Lead levels in drinking-water are still of concern in some areas where there is contamination from lead piping; here ratios of lead isotopes in blood or teeth may give a useful indication of source.

Table 3.2.2 Currently available biomarkers

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Toxic metals							
Lead	Blood lead	Blood	Weeks	+	+++	Established	EQA[c]
	ZPP	Blood	Weeks	+	+++	Established	
	ALAD	Erythrocytes	Weeks	++	++	Established	
	ALA	Urine	Weeks	++	+	Established in workplace	
	Bone lead	*In vivo* bone	Months/years	+++	+	Workable	
	Isotopic ratios	Blood Teeth	Months/years	+++	++	Research	Supplies information about source of lead
Cadmium[d]	Blood cadmium	Blood	Months/years	+	+++	Established	EQA
	Cadmium in urine	Urine	Months/years	+	+++	Established	
	Urinary proteins	Urine	Months/years	++	++	Useful effect marker	RBP rather than β_2- microglobulin
	Urinary enzymes	Urine	Months/years	+	++	Useful effect marker	
	Neutron activation analysis	*In vivo* liver and kidney	Years (decades)	+++	++	Research but well validated	
Mercury	Blood mercury	Blood	Days/weeks	+	++	Workable	
	Mercury in Urine	Urine	Months	+	+++	Established	EQA
Arsenic	Arsenic in urine	Urine	Days	+++	++	Workable	Speciation is required to separate organic arsenic (from diet) from inorganic arsenic
Nickel	Nickel in urine	Urine	Days/weeks [e]	++	++	Workable	
Chromium	Chromium in urine	Urine	Days/weeks [e]	++	++	Workable	

Table 3.2.2 (cont.)

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Aluminium	Aluminium in serum	Blood	Weeks/months	++	++	Established	EQA: Contamination is a major problem, sample collection techniques are crucial
Manganese	Manganese in blood	Blood	Months	++	+	Workable	Contamination during collection is possible
Volatile organic compounds							
Volatile organic compounds in general	Solvents	Breath	Days	+++	+	Research	Direct analysis (mass spectrometry) or collection in the field (technical problems)
Aliphatic hydrocarbons	Solvent	Breath/blood	Days/weeks	+	++	Workable	
	or metabolite	Urine	Days/weeks	+	++	Workable	
Butadiene	Hb adducts	Blood	Weeks/months	++++	+	Under development	Adduct levels are low
	^{32}P postlabelling	Blood	Months	+++		Under development	
Benzene	Benzene	Breath/blood	Days	++	++	Workable	Higher in smokers
	trans, trans - Muconic acid	Urine	Days	++	++	Workable	
	Phenylmercapturic acid	Urine	Days	++	++	Promising	
	DNA-Hb adducts	Blood	Months	+++	+	Not yet useful	
	Phenol	Urine					Insensitive to levels arising from environmental exposure

Table 3.2.2 (cont.)

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Toluene, xylenes, styrene	Solvent	Breath/blood	Days	+	++	Workable	
	Metabolite (e.g. methylhippuric acid, mandelic acid)	Urine	Days	+	+	Workable	High endogenous production of hippuric acid from toluene makes this unsuitable
Chlorinated organic compounds							
Dichloromethane	Parent compound	Breath/blood	Days	++	++	Established	
	Metabolite i.e. CO (i.e. COHb)	Breath/blood	Days/weeks	+	+++	Established	
Chloroform	Parent compound	Breath/blood	Days/weeks	+	++	Workable	
Carbon tetrachloride	Parent compound	Breath/blood	Days/weeks	+	++	Workable	
Trichloroethane	Parent compound	Breath/blood	Days/weeks	+	++	Workable	
Tetrachloroethane	Parent compound	Breath/blood	Days/weeks	+	++	Workable	Some accumulation in adipose tissue
Trichloroethylene	Parent compound	Breath/blood	Days/weeks	+	++	Workable	
	Metabolites	Urine	Days/weeks	+	+++	Established in the workplace	
Lindane	Parent compound	Blood	Days/weeks	++	++	Established	
Dieldrin	Parent compound	Blood	Months/years	++	++	Workable	
Dioxins (also PCBs and PCDFs)	Parent compounds in blood	Blood (GC-MS)	Months/years	++++	+	Established	Chemical species specific
		Blood–CALUX assay (AhR–linked gene assay)	Months/years	++	++	Under validation	For ‘dioxin-like’ chemicals

Table 3.2.2 (cont.)

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Other organic compounds							
PAHs [d]	Hydroxypyrene	Urine	Days	+	+++	Established in the workplace	Ratio of hydroxypyrene to total PAH varies with the source
	DNA and protein adducts	Blood/lung	Days/months	++++	+	Workable	
	HPRT mutations	Blood	Months/years	++++	+	Research	
	^{32}P postlabelling	Blood/lung	Days/weeks	+++	++	Workable	
	HPLC-FL	Blood/lung	Days/weeks	+++	++	Workable	
Heterocyclic aromatic amines	Parent compounds and metabolites, conjugates	Urine	Days	+++	++	Workable/ under development	Foodborne exposures are very low
	DNA adducts	Biopsy tissues	Days/weeks	+++	++	Workable/ under development	High sensitivity required
	Protein adducts	Blood	Weeks	+++	++	Under development	High sensitivity required
Phenol	Phenol	Urine	Days	++	++		Low sensitivity, endogenous production
Organophosphorus pesticides	Metabolites e.g. alkyl phosphates	Urine	Days	++	++	Workable	
	Cholinesterases	Erythrocytes, plasma, whole blood	Days	+	+	Well established	Sensitive to high exposure only
	DNA/protein adducts	Possibly blood	Weeks	+++	+++	Needs development	Development of assay for adducts in blood should be possible

Table 3.2.2 (Cont.)

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Inorganic gases and particulates							
ETS	CO exhaled,	Breath	Days/weeks	++	++	Established	Do not distinguish ETS
	COHb	Blood		+	++		CO from CO from other sources
	Thiocyanates	Plasma,urine, saliva	Weeks	+	+++		Affected by diet
	Nitrosamines		Days	++	+		Poor sensitivity
	Nicotine	Saliva, urine	Days	+	+++	Established	Low sensitivity
	Cotinine	Saliva, urine	Days	+	+++	Established	Widely used
	DNA adducts	Blood	Weeks/months	++++	+	Poor sensitivity	Research
	Hb adducts of aminobiphenyl	Blood	Weeks/months	+++	+	Under development	Research
Nitrogen oxides	None available						
Fine particulates (PM_{10} fraction)	None available						

Table 3.2.2 (cont.)

Exposure	Biomarker	Source of specimen	Time-scale of exposure	Cost[a]	Ease of analysis[b]	State of development	Comment
Other							
Nitrate	Nitrate	24-hour urine	Hours	+	+	Workable	
	Nitrite	Saliva/gastric juice	Hours	+	+	Workable	
	Total *N*-nitroso compounds	Gastric juice	Hours	+++	++	Workable	
	N-nitrosoproline	Urine (24-hour/ overnight)	Days		++	Workable	
	DNA adducts/ deamination	Leucocytes, exfoliated cells, biopsies	Days/weeks		++	Workable	
	Endogenous NO synthase	Tissues	Days/weeks		++	Workable (commercially available antibodies)	NO synthase raised in inflamed tissues

AhR, receptor; ALAD, aminolaevulinic acid dehydratase; CALUX, chemically activated luceiferase gene expression; CO, carbon monoxide; COHb, carboxyhaemoglobin; Hb, haemoglobin; GC, gas chromatography; ETS, environmental tobacco smoke; FL, fluorescence detector; HPLC, high-performance liquid chromatography; MS, mass spectrometry; NO, nitric oxide; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; PCDG, polychlorinated dibenzofuran; RBP, retinol binding protein; ZPP, zinc protoporphyrin

[a] Cost: + low to ++++ high

[b] Ease of analysis: + an analysis needing special care or facilities (not generally available); +++ an established analysis (capable of giving good results in most laboratories without undue difficulty)

[c] EQA, external quality assurance schemes available

[d] Affected by smoking

[e] Time-scale from workplace exposure experience

Neutron activation analysis for measurement of cadmium in liver and kidney has been developed as a biomarker of long-term cadmium exposure (Armstrong *et al.,* 1992).

Long-term exposure to manganese in drinking-water is of concern, because of possible neurological effects. The analysis of manganese in blood or in hair can be accomplished by available methods. However, this does not resolve the question of the source of residues, which is particularly relevant as surface contamination may be a problem with hair analysis.

There are currently 36 elements, including aluminium, arsenic, cadmium, copper, iron, lead, manganese, mercury, nickel, tin and zinc, under surveillance in the UK TDS using ICP-MS. The measurement of urinary and faecal excretion of these metals can be a useful biomarker of individual intake, which will supplement the information from dietary analysis. As with lead, stable isotopes of some other elements can be used to determine routes of exposure.

ORGANIC COMPOUNDS

The use of biomarkers for airborne exposure to organic pollutants in the environment is less well established than for metals. The choice of biomarker depends on the extent and pattern of metabolism of the organic compound involved and on the toxicokinetics of the uptake, metabolism and excretion.

Volatile organic compounds

Analytical and sampling techniques are not nearly so well established for volatile organic pollutants as for other lipid soluble persistent substances. Measurement of VOCs in breath has so far required access to sophisticated but transportable mass spectrometry, although simple field methods for sample collection are under development. For some organic compounds, for example benzene, the major metabolites are identical to endogenous metabolites, and their measurement at environmental exposure levels does not distinguish between exogenous and endogenous origins. Toxicokinetics vary greatly; some VOCs have such short half-lives that measurement in breath or blood does not give representative results.

Chlorinated organic compounds

Chlorinated organic compounds, including PCBs and the dioxins, are under surveillance in food in polluted areas. Monitoring exposure to polychlorinated polycyclic compounds by measurement in body fluids, adipose tissue and breast milk is an accepted technique for individual biomarkers of exposure. The analytical complexity of the techniques involved means that only a few samples can be processed and population studies may become exceedingly expensive. However, several biomarker systems have been developed which may reflect the sum toxicity of complex PCDD, PCDF and PCB mixtures, (see p52).

Pesticides

Pesticide residues in the main food groups are under surveillance in the UK. A number of approaches have been used to monitor human exposure to low levels of pesticides.

Parent compound excretion studies have been performed following experimental exposure of human volunteers to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and other phenoxyacid herbicides, paraquat, and organophosphorus and carbamate insecticides. Organochlorine pesticides, for example dicofol, are excreted more in faeces than in urine (Nigg & Stamper, 1989). Parathion administered to humans was recovered in urine as *p*-nitrophenol and alkylphosphate; 50% was recovered in 24 hours. For the phenoxyacid herbicide MCPA, 40% was excreted in 24 hours and 90% in 72 hours. The current methods are directed at occupational exposure and therefore sensitivity requires further improvement if similar methods are to be used for dietary exposure. Examples of the current sensitivities of methods for metabolites are: 0.01 ppm for chlorpyrifos, 0.1 ppm for benomyl and 0.2 ppm for carbaryl, and for parent compounds: 0.04 ppm for 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T (Lavy & Mattice, 1989).

Haemoglobin protein conjugates have been evaluated for use in monitoring occupational exposure using parent compounds and metabolites (Lewalter & Koralius, 1986). Exposure to known genotoxic pesticides or their metabolites, e.g. dichlorvos and the *N*-nitro derivatives of carbofuran and aldicarb, may potentially be monitored as DNA adducts in lymphocytes (Hallenbeck & Cunningham-Burns, 1985). Both these methods require considerable development if they are to be used to monitor dietary exposure.

Other organic compounds

Air pollution from the combustion of fossil fuels in industry, the home and in the form of petrol or diesel fuel results in human exposure to PAHs. A biomonitoring approach that has proved useful in monitoring occupational exposure is the measurement of the urinary metabolite of pyrene (1-hydroxypyrene). The assumption is made that the ratio of pyrene to total PAHs is constant in any one occupational situation. The measurement of 1-hydroxypyrene is now being investigated for non-occupational exposures. The measurement of exposures to complex but varying mixtures such as PAHs is aided by the identification of a single analyte that represents the uptake behaviour of the whole mixture; this has to be established in any new situation.

There is considerable research experience in using DNA and protein adducts for monitoring PAH exposure in the workplace and ambient environment (Phillips *et al.,* 1993). Collaborative exercises are allowing comparison of results from one laboratory to another, but the technical complexity and low throughput of these assays make large population studies difficult to mount at the present time.

The average daily consumption of benzo[*a*]pyrene in food is less than 1 µg per day in the UK. Surveillance in the UK TDS shows cereals and fats to be the main sources. However, wide individual variation is likely depending on cooking practices and the site of origin of the vegetable source. Hence biomarkers may be useful in assessing individual exposure. Immediate exposure is indicated by 24-hours urine samples (*via* excretion of 1-hydroxypyrene). For longer-term exposures, measures of bulky lymphocyte adducts by ^{32}P post labelling are possible; however smoking and occupational exposures are likely to be major confounders (Lioy *et al*., 1988). Measuring exposure using mutations in certain genetic loci is too insensitive.

The average daily dietary consumption of HAAs in the UK is 20–50 µg per day. However, wide individual variation is likely depending on cooking practices, especially temperature and heat source (fried, grilled, broiled, roasted) and use of pan residues and, as with benzo[*a*]pyrene, biomarkers of individual exposure would be extremely useful. Excretion of parent compounds and metabolites in 24-hour urine samples indicates immediate exposure, and relationships between dose and biomarker measurement have been demonstrated. Adducts of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) and MeIQx (2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline] have been detected in colonic biopsies (Friesen *et al*., 1994); a less invasive approach using exfoliated faecal cells has not been examined.

Biomonitoring techniques for many of the organic pollutants involve the measurement of a metabolite, conjugate or adduct of the pollutant. Genetic polymorphisms in metabolising enzymes not only affect susceptibility (see p65), but are responsible for the varying levels and patterns of metabolites detected in different individuals exposed to the same level of pollutant. An example of this is the effect of acetylator genotype on the urinary excretion of various aromatic amines. Should there be major differences in metabolite or adduct production due to genetic differences, and if the polymorphism is not equally distributed throughout the study population, there will be difficulties in using measured metabolite or adduct levels as an exposure measure. However, for some pollutants analytical methods can be devised that sum the metabolites produced so that the total represents an exposure measure irrespective of the actual contribution of individual compounds to that total. The number of overlapping polymorphisms in cytochrome P450 genes is such that, although individual polymorphisms can give huge differences at the enzyme level, the actual range of metabolite production from individual to individual is very much less and will not necessarily be a problem in exposure studies. However, these possible confounding effects should be kept in mind.

INORGANIC GASES AND PARTICULATES

Although there is considerable concern about ambient and indoor air pollution by inorganic gases and particulates, there are few biomarkers available or under development for assessing exposure. The exceptions are the use of carboxyhaemoglobin and breath CO for CO exposure, which are well established techniques. No satisfactory method is available for NO_x, SO_2, or O_3. In contrast, exposure to environmental tobacco smoke, with its content of organic compounds, can be monitored using several biomarkers, of which cotinine in urine or saliva (Jarvis, 1987) is the best validated.

Air pollution epidemiology studies have recently indicated the importance of exposure to fine particulates (PM_{10} and ultrafine particles; e.g. Dockery *et al.,* 1993; Pope *et al.,* 1995). Epidemiological studies of air pollution, either episodic or background, would be greatly assisted by some biological measure of individual exposure. However, although there is some knowledge of the composition of these particles, no satisfactory biomonitoring technique is available.

OTHER AGENTS

There would appear to be little point in searching for biomarkers of nitrate exposure from food, since the main human sources of nitrate are endogenous production of nitric oxide from arginine *via* nitric oxide synthase (inducible and constitutive; Liu & Hotchkiss, 1995) and nitrate in water. Nitrate levels in vegetables are under surveillance by the Ministry of Agriculture, Fisheries and Food. One of the main concerns about nitrate exposure has been the possibility of endogenous *N*-nitrosation, and a number of approaches have been used to measure this pathway (see Table 3.2.2).

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4 Conclusions and recommendations

There is a major need for more and better methods of assessing exposure to environmental pollutants. These would have application in epidemiological studies of both short- and long-term risks to health, whether they are caused by short-term pollution incidents or long-term exposures.

Investigation of the immediate and long-term health effects of acute pollution episodes such as those associated with accidental exposures to aluminium sulphate in drinking-water (Camelford, UK), dioxins (Seveso, Italy) or methylisocyanate (Bhopal, India) would have been aided if an appropriate biomarker had been available to reflect the extent of exposure and if the relevant samples had been collected.

For long-term exposures, two approaches are possible. In the first, exposures are assessed retrospectively in subjects who have developed the disease of interest and in controls who do not have the disease. If a biomarker is to be used for this purpose it must indicate long-term exposure and must not be modified by the occurrence of the disease. A few such biomarkers are already available and in use. For example, levels of DDT in breast fat and blood have been compared in cases of breast cancer and controls. Other techniques, such as neutron activation analysis for measurement of cadmium in liver and kidney have also been developed, but there are many pollutants for which no suitable method will be available in the immediate future. The development of further biomarkers for retrospective assessment should therefore be a long-term research aim.

The alternative approach is to assess exposure prospectively and relate it to subsequent disease incidence. This method avoids the bias that can occur if the disease process modifies the level of a biomarker, but can be statistically inefficient if only a small proportion of subjects develop the disease of interest, and may take many years to produce useful results. One possibility is to adopt a nested

case–control design in which tissue samples are collected and stored prospectively, but are only analysed for the subjects who subsequently develop the disease of interest and for a sample of controls. This considerably reduces the number of assays required, but is only possible if the measurement is not affected by sample storage. Suitable assays are currently available for most toxic metals and some organic compounds, and the techniques that are being developed for measurement of DNA and haemoglobin adducts open up exciting new possibilities. A drawback, however, is the cost and the limited scale on which many of the tests can currently be carried out.

There is an urgent need to establish the effect of prolonged sample storage on biomarkers that might be used in this way, especially DNA and haemoglobin adducts. Such biomarkers will be particularly useful if they can be reliably measured in blood, serum or plasma that has been routinely stored at -20 °C, since samples of this sort are already available from a number of ongoing studies.

Where the relevant exposures are chronic, as is usually the case, an index of longer-term exposure (over months or more) is normally required. However, in the recently completed nested case-control study of risk factors for hepatocellular carcinoma in China, a measure of urinary aflatoxin metabolites in a single spot urine sample collected at the time of recruitment into the study cohort was highly predictive of the eventual disease outcome.

When tissue samples are collected and stored as part of a prospective study, or immediately after an acute exposure incident, it may not always be clear what assays will eventually be required, and some tests may be sensitive to contamination of sampling equipment or containers. It is therefore sensible to retain unused sampling equipment (needles, syringes, etc.) and containers, so that checks for possible contamination can be made if required.

Even where biomarkers cannot be used to assess exposure directly in epidemiological studies, they may be useful in the validation of other means of exposure assessment. For example, studies using breath concentrations of benzene as a marker for recent uptake have established smoking, filling cars with petrol, driving in heavy traffic and environmental tobacco smoke as important contributors to individual dose. This suggests areas that should be covered in questionnaires seeking to assess benzene exposure. Identifying the sources of exposure and understanding the determinants of individual uptake of pollutants are important factors in the design of control strategies. There is little point in investing substantial resources to reduce levels of a pollutant in air if the main

source of exposure is drinking-water. Biomarkers have a central role in the investigation of factors influencing individual uptake, and as yet have not been adequately exploited for this purpose. For many pollutants, suitable short-term or medium-term biomarkers are already available for such studies.

The other major application of biomarkers of exposure is in population monitoring to assess the need for, and response to, environmental control measures. For this purpose measurements must usually be carried out in large numbers of people and therefore need to be inexpensive and non-invasive. A few suitable biomarkers are currently available, e.g. urinary levels of mercury and cadmium, and others could be developed, such as a less costly and more widely available method for the measurement of benzene in breath. For many of the major air pollutants, however, personal air monitoring is, so far, more practical than the use of biomarkers.

Where biomarkers are used of population monitoring it is especially important that they are subject to adequate quality control and assurance procedures. For example, there is a need for more inter-laboratory comparison of assays for DNA and haemoglobin adducts.

Finally, more research is needed on the genetic polymorphisms and other constitutional factors that modify levels of biomarkers in response to a given environmental exposure. Useful information may come not only from studies in people, but also from experiments on laboratory animals in which genetic differences can be more rigorously controlled.

OVERALL RECOMMENDATIONS

- The development of new biomarkers as measures of long-term exposure to pollutants should be encouraged, not only for pollutants that are known or suspected carcinogens, but also for neurotoxins and developmental toxins and hormonally active substances.
- Currently available biomarkers should be used more widely to assess the sources of exposure that influence personal uptake of pollutants.
- More attention should be given to quality control and standardisation of assays for biomarkers.

- There is an urgent need to establish the effect of sample storage on biomarkers that might be used in nested case–control studies.

- If tissue samples are collected and stored as part of a prospective epidemiological study, unused sampling equipment and containers should be retained for future checks on possible contamination.

- More research is needed on genetic polymorphisms and other constitutional factors that modify biomarker responses to environmental exposures.

LIST OF PARTICIPANTS

WORKSHOP ON THE USE OF BIOMARKERS IN ENVIRONMENTAL EXPOSURE ASSESSMENT

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Members

Dr D Benford, The Robens Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey

Dr S Bingham, MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge (*Session Chairman*)

Dr A Brouwer, University of Wageningen, PO Box 238, 7600 AE Wageningen, The Netherlands

Professor J Caldwell, Department of Pharmacology and Toxicology, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG

Professor P Calow, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN (*Session Chairman*)

Dr D Coggon, MRC Environmental Epidemiology Unit, Southampton General Hospital, Southampton SO9 4XY (*Meeting Chairman*)

Dr H Crews, CSL Food Science Laboratory, Norwich Research Park, Colney, Norwich NR4 7UQ

Dr HT Delves, SAT Trace Element Unit, University Department of Clinical Biochemistry, Southampton General Hospital, Tremona Road, Southampton SO16 6YD

Professor M Depledge, Plymouth Environmental Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA

Dr P Farmer, MRC Toxicology Unit, Hodgkin Building, University of Leicester, Lancaster Road, Leicester LE1 9HN

Mr J Fawell, Water Research Centre plc, Henley Road, Medmenham, Marlow, Bucks. SL7 2HD

Dr V Forbes, Department of Biology and Chemistry, Roskilde University, PO Box 260, DK4000 Roskilde, Denmark

Professor D Forman, Centre for Cancer Research, University of Leeds, Arthington House, Cookridge Hospital, Leeds LS16 6QB

Professor RC Garner, The Jack Birch Unit, Biology Department, University of York, Heslington, York YO1 5DD

Dr D Gompertz, Institute for Environment and Health, University of Leicester, 97 Regent Road, Leicester LE1 7DD (*Session Rapporteur, Consultant Editor*)

Dr N King, 'The Gyles', Bledlow Road, Saunderton, Princes Risborough, Bucks. HP7 9NG (*Session Rapporteur*)

Mr C Livesey, Central Veterinary Laboratories, New Haw, Addleston, Surrey KT15 3NB

Mr H Mason, Health and Safety Laboratory, Broad Lane, Sheffield S3 7HQ

Dr P Matthiessen, MAFF Fisheries Laboratory, Burnham-on-Crouch, Essex CM10 8HA

Professor AJ McMichael, London School of Hygiene and Tropical Medicine, Department of Epidemiology and Population Science, Keppel Street, London WC1E 7HT (*Session Chairman*)

Professor M Moore, Plymouth Marine Laboratory, Citadel Hill, Plymouth PL1 2PB

Dr D Osborn, Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon PE17 2LS

Dr D Peakall, Monitoring and Assessment Research Centre, King's College, Campden Hill, London W8 7AD

Dr D Phillips, Haddow Laboratories, Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG

Dr P Schulte, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, USA

Dr D Shuker, MRC Toxicology Unit, Hodgkin Building, University of Leicester, Lancaster Road, Leicester LE1 9HN (*Session Rapporteur*)

Dr H Thompson, MAFF CSL, London Road, Slough, Berks SL3 7HJ

Professor J Timbrell, The School of Pharmacy, Department of Toxicology, University of London, 29/39 Brunswick Square, London WC1N 1AX

Professor P Toniolo, New York University School of Medicine, 341 East 25th Street, New York NY 10010-2598, USA (*Session Chairman*)

Professor C Walker, Department of Physiology and Biochemistry, AMS Building, Reading University, Whiteknights, Reading RG6 2AJ (*Session Rapporteur*)

Dr K Wilson, Robens Building, Health and Safety Laboratory, Broad Lane, Sheffield S3 7HQ

Representatives

Dr K Cameron
Dr R McDanell
Dr L Smith
Department of the Environment, Romney House, Marsham Street, London SW1P 3PY

Dr R Maynard
Dr M Waring
Department of Health, Skipton House, London Road, London SE1 6LW

Dr D Tennant, MAFF Food Science Division, Nobel House, 17, Smith Square, London SW1P 3JR

Dr M Kemp, MRC Head Office, 20 Park Crescent, London W1N 4AL

IEH Secretariat

Dr P Harrison (*Programme Manager*)
Dr C Humfrey
Dr L Shuker (*Head of Scientific Publications Unit*)

Technical assistance

Mrs P Forster
Miss S Howe

Acknowledgement

Editing by Sheila Poole, Birmingham

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Natural and Man-Made Mineral Fibres: UK Research Priorities

Report R3 published 1995

Perinatal Developmental Neurotoxicity

Report R4 in preparation

The Use of Biomarkers in Environmental Exposure Assessment

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IEH Assessments

Environmental Oestrogens: Consequences to Human Health and Wildlife

Assessment A1 published 1995

Indoor Air Quality in the Home: Nitrogen Dioxide, Formaldehyde, Volatile Organic Compounds, House Dust Mites, Fungi and Bacteria

Assessment A2 published 1996

Special Report

Understanding Asthma

Published 1995

For further details please contact:

IEH, University of Leicester, 94 Regent Road Leicester LE1 7DD
Phone 0116 223 1600 Fax 0116 223 1601