BIOMARKERS, VOL. 9, NO. 3 (MAY–JUNE 2004), 211–242

Taylor & Francis
healthsciences

REVIEW
Role of biomarkers in monitoring exposures to chemicals: present position, future prospects

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Received 5 April 2004, revised form accepted 27 September 2004

Biomarkers are becoming increasingly important in toxicology and human health. Many research groups are carrying out studies to develop biomarkers of exposure to chemicals and apply these for human monitoring. There is considerable interest in the use and application of biomarkers to identify the nature and amounts of chemical exposures in occupational and environmental situations. Major research goals are to develop and validate biomarkers that reflect specific exposures and permit the prediction of the risk of disease in individuals and groups. One important objective is to prevent human cancer. This review presents a commentary and consensus views about the major developments on biomarkers for monitoring human exposure to chemicals. A particular emphasis is on monitoring exposures to carcinogens. Significant developments in the areas of new and existing biomarkers, analytical methodologies, validation studies and field trials together with auditing and quality assessment of data are discussed. New developments in the relatively young field of toxicogenomics possibly leading to the identification of individual susceptibility to both cancer and non-cancer endpoints are also considered. The construction and development of reliable databases that integrate information from genomic and proteomic research programmes should offer a promising future for the application of these technologies in the prediction of risks and prevention of diseases related to chemical exposures. Currently adducts of chemicals with macromolecules are important and useful biomarkers especially for certain individual chemicals where there are incidences of occupational exposure. For monitoring exposure to genotoxic compounds protein adducts, such as those formed with haemoglobin, are considered effective biomarkers for determining individual exposure doses of reactive chemicals. For other organic chemicals, the excreted urinary metabolites can also give a useful and complementary indication of exposure for acute exposures. These methods have revealed 'backgrounds' in people not knowingly exposed to chemicals and the sources and significance of these need to be determined, particularly in the context of their contribution to background health risks.

Keywords: biomarkers, biomonitoring, chemical exposure.

Introduction

There is considerable interest in the use of biomarkers in research and occupational and environmental toxicology. This has been reflected in a number of reviews on biomarkers that have highlighted the important role that biomarkers can play in contemporary toxicology (Timbrell 1998, Waterfield and Timbrell 1999, Waterfield 1999, De Caprio 1999, Mutti 1989, 1999). The principal aim of this review is to review the major developments that have occurred in academic, institutional and industrial organizations working on biomarkers for monitoring human exposure to chemicals. The most significant developments for new and

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Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 2004 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/13547500400015642

existing biomarkers, analytical methodologies, validation studies and field trials, and auditing and quality assessment of data are discussed. The developments in chemical-specific biomarkers for occupational and environmental exposure to chemicals that reflect specific exposures and possibly permit the prediction of the risk of disease are also reviewed. The views of practitioners were counselled to judge the prospects of research and applications of biomarkers for monitoring human exposures, the associated ethical issues, and the handling of generated data and its application in risk assessments. A particular emphasis has been made on cancer biomarkers and their potential for predicting adverse toxicological outcomes.

Many research groups in Europe and elsewhere are carrying out studies on the development of biomarkers and their applications for human and environmental monitoring (Wilson and Suk 2002). Major goals of many of the research programmes are to develop and validate biomarkers that reflect specific exposures and permit the prediction of the risk of disease in individuals and population groups (Mutti 1995, Groopman and Kensler 1999, Bartsch 2000, Trull *et al.* 2002). From results of studies on inter-individual variability, it has become evident that each individual is likely to have a unique response to the exposure dose and the time to the onset of disease (IARC 1999b). Nevertheless, the minimum expectation is that biomarkers should be able to classify the exposure status of individuals and groups of populations. Although conventional epidemiology has identified smoking as a major cause of human cancer (Doll 1996), means for the quantitative determination of exposure are especially important because the ability to define the amounts and nature of the exposures is often a major difficulty in conventional epidemiology studies (Ehrenberg and Törnqvist 1992, Ehrenberg *et al.* 1996).

In the newer and emerging discipline of molecular epidemiology, the precise determination of individual exposure to chemicals, especially carcinogens, is considered a highly desirable goal (Groopman and Kensler 1999). In the case of molecular cancer epidemiology, improved detection and prevention have been viewed as long-standing principal goals (Bartsch 2000, Albertini 2001). Advocates of molecular epidemiology indicate that it has significant potential in preventing cancer and other diseases caused by exposures related to lifestyle, occupation or ambient pollution (Perera and Whyatt, 1994, Perera 1995, Perera *et al.* 1996, Perera 2000, Perera and Weinstein 2000, Wang *et al.* 2001). Biomarkers play a key role in these investigations but it has been pointed out (Perera 2000) that many studies failed to use validated biomarkers or used designs that did not adequately consider the biology of the endpoints. Successful studies could have major economic impact in modern societies. For example it has been estimated that prevention of 20% of the cancers in the USA would result in 200 000 fewer cases diagnosed each year and in annual savings of US$21.4 billion in direct costs alone (Perera 2000).

Concern about increasing cancer amongst children has made protecting children's health the US Environmental Protection agency's highest priority (Goldman 1998, Carroquino *et al.* 1998, Claudio *et al.* 1998). There have been suggestions that young children and the developing foetus may be more susceptible to effects of environmental toxicants than adults due to differential exposure patterns and developmental immaturities (Whyatt and Perera 1995). Molecular

epidemiological approaches may play an important role in addressing the concerns about the effect of chemicals on children and the unborn (Perera *et al.* 2002).

Viewed overall, biomonitoring for carcinogen exposure has represented the major proportion of efforts in research and application of biomarkers. Some commentators have drawn attention to the potential for bias in molecular epidemiology studies (Vineis and McMichael 1998) and, naturally, others to the limitations of biomarkers of exposure in cancer epidemiology (Pearce *et al.* 1995). A very recent example of the value of human biomonitoring was demonstrated by the identification of human exposures to acrylamide from the diet, which subsequently led to the identification of the source of this carcinogen in foodstuffs, especially carbohydrates, notably potatoes, cooked at high temperatures (Tareke *et al.* 2002, Mottram *et al.* 2002, Stadler *et al.* 2002, Yasuhara *et al.* 2003, Yaylayan *et al.* 2003).

Recent research studies have also focussed on specific early effect markers such as certain oncogenes and tumour suppressor genes (Vainio 2001). Together with advances in molecular genetics, this has led to much interest in susceptibility factors and identification of polymorphisms of various enzymes (Bartsch *et al.* 1998b). Studies involving biomarker measurements have been carried out in at risk cancer groups based on epidemiological results relating to exposures many years earlier (Bartsch 2000). New epidemiological studies are unable to address the effective exposures after about the mid-1970s and therefore biomarker and biomonitoring studies could be used for monitoring current exposure levels. Studies relating phenotype/genotype with cancer have examined measurable early endpoints such as DNA adduct formation and/or cytogenetic damage (Godschalk *et al.* 2001).

There is considerable inter-individual human variation in levels of adducts formed from environmental and occupational exposures to chemicals. It is evident that groups of predisposing polymorphic genes exist, for example, in those involved in metabolism and DNA repair (Bartsch *et al.* 1998a, IARC 1999b). Biomonitoring and molecular epidemiology could therefore play an important role in identifying susceptible individuals, particularly those suffering a combination of high risk factors, namely a high level of exposure to chemicals, inherited cancer predisposing genes and a deficiency of protective factors such as can occur due to the diet. Individual susceptibility factors can influence all the stages between exposure and the onset of disease (figure 1). Once identified, predisposing genes, e.g. for cancer, might be used as intermediate risk markers for disease.

This present review has especially aimed at identifying which of the existing biomarkers provide reliable data that could be used for risk assessment and which of the newly developed biomarkers hold promise for monitoring exposure and assessing risks. The various markers and their classification as biomarkers of

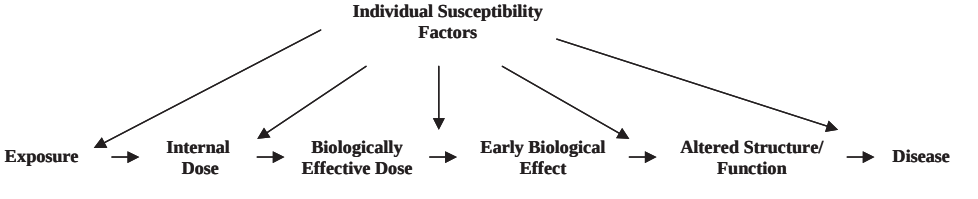

Figure 1. Stages linking exposure to chemicals with clinical disease.

exposure or effect and which may represent the different stages resulting from exposure and leading to disease are shown in figure 2. Analytical methodologies for existing biomarkers in use for human monitoring have been evaluated, and where further developments are necessary these have been identified. The adequacy of validation studies carried out so far and quality assurance procedures in place for human studies have also been reviewed. The ethical issues arising from human biomonitoring studies and the handling of the generated data are discussed in addition to possible future directions for biomarker research.

Biomarkers of exposure

Biomarkers of exposure are exogenous substances, metabolites, the product of an interaction between a xenobiotic and a target molecule or cell measured within a compartment of an organism (NRC 1989). The terms 'biomarker of exposure' and 'biological indicator of internal dose' have essentially the same meaning but the latter expression emphasizes its relationship with adverse effects in a critical organ or tissue. Biological monitoring and molecular dosimetry measurements are terms referring to the periodic measurement of a biomarker to assess the health risk associated with exposure to a chemical (Groopman and Skipper 1991). Whilst molecular dosimetry embodies the concept of quantitative determinations, biological monitoring refers to the process of a regular basis of measurements (Mutti 1999).

It should be noted that several biomarkers may be available for the same chemical. In addition, the meaning of the marker may depend on the sampling time. For example in the case of markers with a short half-life, e.g. compounds measured in blood, the internal dose may mean the amount of chemical absorbed shortly before the biomonitoring sample was taken. For markers with intermediate half-life, e.g. urinary metabolites, the internal dose, which can be estimated, is that occurring during the preceding days. For markers with long half-life, e.g. adducts to DNA or haemoglobin, the internal dose is integrated over a period of months. For chemicals that accumulate, the internal dose refers to the amounts stored in tissues and organs over an extended period of years. Where an interaction occurs between a reactive metabolite and a critical molecular target, this represents a biologically effective or target dose. However, the true target is usually not accessible and

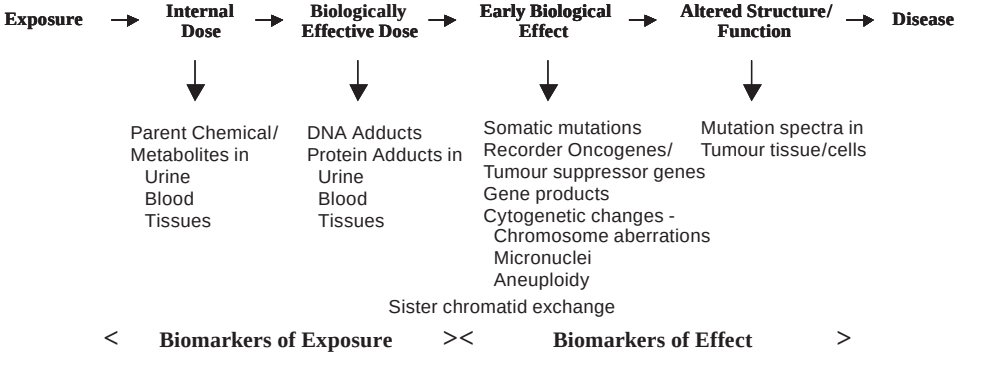

Figure 2. Relation of events and biomarker classification (Albertini 2001).

biomarkers of exposure are surrogate measures of something impossible to measure. Whilst most biomarkers reflect primarily either exposure or the effect of the exposure, there can be a continuous relationship between biomarker of exposure and biomarker of effect, and, the use of biomarkers, rather than their intrinsic properties, may define their classification (figure 2). In the case of DNA adducts, although these are generally considered as biomarkers of exposure, their formation by interaction with a cellular macromolecule is also indicative of an effect. Moreover, their persistence depends on individual repair capability and hence DNA adducts may also be regarded as biomarkers of susceptibility. The time of sampling of a biomarker may give different meanings to the marker. Therefore, knowledge of the mechanism of the end-point to be measured is especially important in order to permit selection of a relevant marker of internal dose and appropriate sampling times.

Application of biomarkers in exposure assessment

The principal aims of biomarkers in exposure assessments are to establish unequivocally the fact of exposure in population studies, reduce or prevent misclassification in epidemiological studies, and, to determine the internal dose that occurs in a critical organ, cell or molecule. Irrespective of the aim, biomarkers of exposure allow a focus on the body burden or the total absorbed dose and integrated sources and routes of exposure, patterns of exposure, and, inherent and behavioural differences between individuals. If these factors are not considered important then biological monitoring may be unnecessary because it may add variability to the alternative of ambient atmosphere monitoring (Mutti 1999).

Between individuals, the same biomarker may vary both as a function of time, and at a given time, between individuals exposed to the same air concentrations. The kinetics of the disposition of the exposure chemical are important in respect of possible biological variability relative to exposure (Droz 1993). Thus, biomonitoring is normally not feasible for biomarkers with a half-life less than 2 h. For half-lives in the range 2–10 h a sample taken at the end of a working day represents the integrated exposure during the work day. In the case of half-lives of between 10 and 100 h the optimal time for sampling is at the end of the 5-day working week and the determinations reflect exposure during the preceding few days (UK HSE 1992). For the monitoring of chemicals that have long half-lives, it is generally agreed that biomarkers of exposure have considerable advantages due to stability and require relatively few measurements to define exposure compared with measurements based on air levels (Rappaport 1995).

Rather than being a complication, the variations seen between and within individuals are valuable in the determination of risk. A particular value of biological monitoring is that it helps to explain variance of data and the choice of a particular biomarker, instead of others. It should not be seen as a means to reduce variance, which always occurs in human populations. Obviously there is a need to standardize collection procedures and analytical methodology to maintain as low as possible variance in these components, but inter-individual differences in the rates

of uptake, biotransformation and excretion need to be emphasized in order to use the data for assessing and managing the associated health risks.

Considerations of kinetics and choice of biomarker

A range of biomarkers may be available for a particular substance, including the parent compound or its metabolites in body fluids such as blood, serum and urine or in accessible tissues such as hair, the adducts of reactive metabolites to DNA or haemoglobin or albumin. Methods for heavy metals may employ estimates of concentrations in critical or storage tissue such as kidney cortex or bone. Selection of the most appropriate approach depends on the mechanistic basis of the adverse effect. These may be classified as acute or chronic, local or systemic, early or delayed, reversible or non-reversible, thresholded or non-thresholded.

Although for acute or local effects, biomarkers of exposure may not be useful for preventative purposes, successful attempts to develop biomarkers of target tissue dose and effect for toxic elements acting on the lung, the largest surface of the body in contact with ambient air, have been reported in workers from the hard metal industry (Goldoni *et al.* 2004).

Chronic and irreversible effects can result from either cumulative doses or cumulative effects. Similar cumulated doses may result from repeated short-term high doses or from long-term low-level exposures. Often it is not possible to predict which of these patterns is relevant for health risks. A relevant biomarker of exposure should therefore aim to link occupational or environmental exposure with a long-term health effect or a relevant intermediate end-point (Mutti 1995). In situations where the mechanism of toxicity is known the selection of suitable biomarkers for the observed effects can be based on kinetic parameters. Knowledge of the toxicokinetics of the chemicals under consideration is an important prerequisite of biological monitoring of exposure (Bernard 1995). In general, it is assumed that biomarkers with longer half-lives correlate better with effects resulting from chronic, long-term, low-level exposures. The half-life is a principal quantitative parameter derived from mathematical models describing the behaviour of xenobiotics in biological systems. It can be derived from a number of sources. These include: fits of experimental data to empirical mathematical formulae, compartmental models which incorporate experimentally determined rate constants, or simulation modelling using physiologically and metabolically based parameters.

There are four main categories of biomarkers of exposure based on their biological half-life. These are defined as: very short, e.g. phenol for benzene exposure, short, e.g. 2,5-hexanedione for hexane exposure, long, e.g. heavy metals such as lead, mercury and cadmium in blood or adducts to DNA and haemoglobin for electrophilic compounds or metabolites, and very long, e.g. heavy metals in bone. The useful time ranges for monitoring urinary metabolites, DNA adducts and haemoglobin adducts following a single exposure are shown in figure 3. For metallic and transition elements, there are often complex metabolic pathways depending on particle size, solubility and oxidation state and species difference for the same element in respect of local and systemic effects as well as biotransformation.

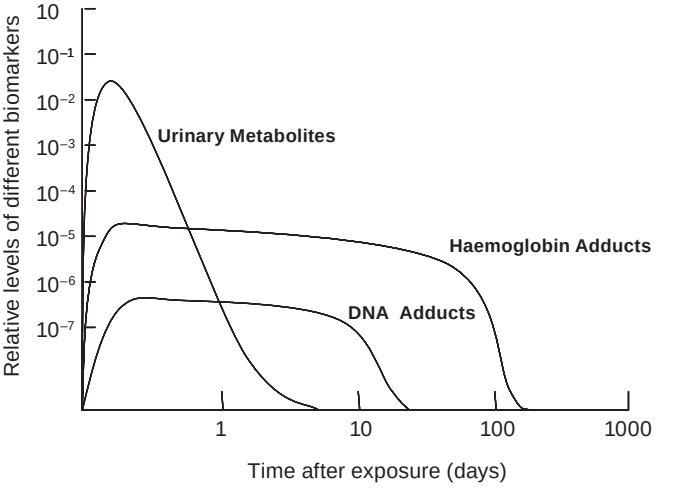

Figure 3. Monitoring time ranges for urinary metabolites, DNA adducts and haemoglobin adducts following a single exposure (Henderson *et al.* 1989).

General suitability of different biomarkers for different classes of chemicals

Genotoxic chemicals

Of the existing more widely used biomarkers, for example, urinary excretion products, haemoglobin adducts, DNA adducts, sister chromatid exchanges (SCEs), micronuclei (MNs), chromosome aberrations (CAs), the consensus view is that in the case of genotoxic chemicals protein adducts are preferred for monitoring long-term exposure and urinary parent compound or metabolites are preferable for monitoring short term exposures. Where chemically specific markers are necessary, adducts or urinary metabolites are certainly preferred. Although cytogenetic markers are long lived they generally have poor specificity and provide no information about the exposure chemical. SCEs are generally considered not useful for monitoring exposures.

Non-genotoxic carcinogens

The monitoring of non-genotoxic carcinogens is more difficult and there are no general markers suitable for all compound classes. The parent compound or excreted urinary metabolites provide the most reliable indication of exposure in the short term. The detection of enzyme induction, induction of cell proliferation or inhibition of gap-junction intercellular communication, or modulation of apoptosis are also viewed as useful indicators. New genomic and or proteomic approaches that are being developed (see below) may have potential in the future as biomarkers for non-genotoxic carcinogens. Protein markers are viewed as being more reliable indicators of effect, although quantification of increments in existing proteins are expected to be difficult. Considerable hurdles are necessary to be overcome, particularly in the area of interpretation of the results of transcript profiling studies (see below).

Neurotoxic chemicals

It is also difficult to generalize on the methods useful for monitoring exposures to neurotoxic chemicals (Manzo *et al.* 1996). Urinary metabolites, such as mercapturates and porphyrins are viewed as having utility. In some specific cases protein adducts may be useful where reactive metabolites are involved, e.g. acrylamide (Hagmar *et al.* 2001). Analyses of hair and blood can be valuable for specific chemicals (MeHg) or blood and breast milk for PCBs. Effect markers that have been used as an indication of exposure are aminolevulinic acid dehydratase (lead), acetylcholinesterase (organophosphates), monoamine oxidase B (styrene, manganese), dopamine beta hydroxylase (manganese, styrene), cholinergic muscarin receptors (organophosphates), serum prolactin (manganese, styrene), calcium (mercury). There are few examples indicating that biomarkers of exposure and effect can be used to predict neurotoxicity based on dose–response relationships in occupationally exposed subjects (Mutti *et al*. 1993, Smargiassi and Mutti 1999).

Developmental/reproductive toxicants

For monitoring exposure to developmental toxicants, which may have potential developmental effects, in some cases measurements of protein adducts are possible in the umbilical cord blood of the newborn. DNA adducts have also been measured in placenta and/or blood and correlations with the amount of smoking have been observed. For monitoring a potential effect on female reproduction, DNA adducts can be determined in oocytes and granulosa in order to assess germ cell damage. For monitoring chemical exposures that may have potential reproductive effects, protein and DNA adducts or DNA strand breaks in sperm are possible indicators.

Studies on adduct formation with macromolecules

Measurements of adducts formed by the reaction of electrophilic metabolites of genotoxic compounds with macromolecules are especially useful because they represent the dose that has escaped the detoxication process and that has reached the macromolecule, e.g., protein or DNA. Methods to quantify adducts from electrophilic compounds were originally developed by Ehrenberg's group in Stockholm in order to determine what was referred to as the 'target' or true dose following an exposure to a genotoxic compound (Ehrenberg *et al.* 1977, Ehrenberg and Osterman Golkar 1980, Wright *et al.* 1988, Watson and Wright 1991). Because red blood cells have a long life of about four months in humans the covalent reaction of compounds with haemoglobin is considered to be a good biomarker for measuring the cumulative internal dose due to repeated exposures (Watson and Van Sittert 1996). It has been considered that this feature of quantitative dose determination would also permit risk assessment for non-cancer endpoints (Costa 1996). Covalent adducts with serum albumin have a shorter lifetime in blood of about 20 days and therefore reflect a more limited period of exposure. One advantage of albumin adducts is that reactive metabolites can interact with this protein in the blood stream without having to penetrate a cell membrane. However, haemoglobin adducts are viewed as a more accurate biomarker if the requirement is for adducts to reflect the cellular level at a target site (Costa 1996, Törnqvist *et al.* 2002).

Macromolecular adducts may also serve as indicators of susceptibility since their quantitative formation and repair probably has a genetic basis. In principle, therefore, adducts formed with macromolecules are close to the ideal in the requirements necessary for risk assessment. Macromolecular adducts provide an integrated measure of exposure, they can represent both early and often reversible biomarkers of effect, and, they reflect individual susceptibility to chemical agents, which can ultimately provide an assessment of the individual risk (Watson and Wright 1991, Watson and Van Sittert 1996). The expectations required of an ideal biomarker of exposure are that it is specific for a particular chemical, detectable and quantifiable in very low quantities, measurable by non-invasive techniques, inexpensive, associated with prior exposure and having predictive capability for a specific disease status (Henderson *et al.* 1989). However, in practice, measurements of adducts are often time consuming, difficult to perform and achieve standardization, and are usually restricted to those compounds or their metabolites that can form covalent products with the nucleophilic centres in macromolecules. Among these, protein adducts seem to be preferable. Protein adducts do not undergo repair and therefore represent less ambiguous biomarkers of exposure as compared to DNA adducts, whose concentration depends not only on formation, but also on their rate of repair.

Knowledge of the mechanisms that lead to the ultimate toxicological endpoint of interest is the main determinant in selection of a relevant biomarker of exposure. A suitable biomarker of exposure should correlate with exposure to a single chemical, but if the exposure can be directly measured then there is a limited scope for biomonitoring. Biomarkers of dose may be more useful to assess the dose effect and dose–response relationships to a chemical. Whilst biomarkers of exposure can clearly define the chemical that caused the exposure the predictive value of a biomarker of dose is determined by its ability to predict adverse toxicological effects (Mutti 1999).

Validity of biomarkers of exposure

The validity of biomarkers of exposure is the degree to which the results of measurements correspond to the end-point or phenomenon being measured. Other terms such as accuracy, sensitivity and specificity need to be considered especially in the context of the application with regard to the ability to predict adverse effects reflected by numbers of the false negatives and false positives found in applications. Relevance and stability are important properties in determining whether a biomarker is suitable for applications in the field. Determinations of concentrations of the parent compound in the biological media are generally preferable to metabolites where the same metabolites may arise from different compounds. In some cases such as in solvent exposure, the parent compound correlates better with exposure compared with metabolites (Ikeda 1999). However, the parent compound itself is often unrelated to the adverse effects that frequently occur due to biotransformation and metabolic activation. For gaining information on dose–response relationships urinary metabolites have often been used successfully as biomarkers of dose (Alexander *et al.* 2002). The analytical validity of biomarkers is determined by their selectivity, range, accuracy and precision. Although simple colorimetric assays can be used in some instances sophisticated chromatographic

and analytical methods are usually necessary. Prior separation of analytes is often necessary and many of the methods rely on the use of mass spectrometric detection because of its selectivity and sensitivity. The measurement of sub-nanomolar concentrations is routinely possible and this has led to the detection of 'backgrounds' with the necessity for stringent controls and a need for their interpretation. In some instances well validated analytical techniques such as gas and liquid chromatography coupled with specific detection and quantification methods such as electron capture and electrochemical detection have sufficient sensitivity and specificity for occupational monitoring and sometimes even for environmental monitoring. For monitoring exposure to metals, atomic absorption spectrometry is useful for the analysis of trace elements in biological fluids, particularly if it is not necessary to discriminate between different compounds of the same element.

For identification of exposure, the chemical itself provides more specific evidence than any of the metabolites, which could come from several sources and sometimes include confounders. The analytical methods available usually permit quantification of both organic and inorganic analytes in the nanomolar range. At such low levels, confounders are likely to occur if variables such as smoking are not taken into account. The local values for controls need to be defined and taken into account since variations can occur due to time and place related environmental pollution. As discussed above, for quantitative exposure assessment, factors such as kinetics should be considered since these may influence the ability of the biomarker to reflect exposure during the selected exposure period. For risk characterization, factors such as toxicodynamics and, where available, the dose–response relationships need to be taken into account. So far, there are only a few accepted biomarkers of dose that can be used to predict adverse effects with a high degree of certainty. Amongst these, are blood lead levels and urinary concentrations of cadmium.

Quality assurance (see also below) is a key issue and a prerequisite for the validation of a biomarker of exposure and in general this needs to be improved in biological monitoring studies (Aitio and Apostoli 1995). The assurance process entails taking measures that results are reliable, that scientifically sound criteria are used in selection of the biomarker, that technically sound practices are used in the collection, storage and analyses and also in the recording, reporting and interpretation of results. Quality control, either internal or more preferably external, is a necessary part of the quality assurance process with the aim of verifying the analytical results of the laboratory. Many studies carried out are research studies of a limited nature and a quality audit is rarely included. A critical assessment of the relationships for exposure–dose, dose–response and effect–disease must be made. For each of these relationships, there may be a possible role for susceptibility markers to identify confounders or modifiers. For short-lived organic compounds, there are many studies confirming the validity for exposure dose but rather fewer for dose–response relationships. For relatively long-lived metals, the opposite appears to be true. For metal toxicology, biomarkers of dose should not necessarily relate to recent exposure but predict adverse effects. Biomonitoring of organic compounds has been used mainly to determine that the internal dose is indicative of exposure. The latter can, in many cases, be

relatively easily measured, and the role of biomarkers as a surrogate of something that is difficult or impossible to measure may therefore be questionable. There are a number of occupations and exposure situations where skin is a major route of absorption. Moreover, with the advent of the greater use of personal protection devices in the workplace, ambient exposure–dose relationships may become difficult to prove and perhaps meaningless. In such situations, the lack of relationships between exposure and biomarkers of dose points to a better definition of the actual absorption judged by biomarkers and should not be used to disprove their validity. Indeed, in these situations there is no credible alternative to biological monitoring as a means to assess actual exposure.

Biomarkers and risk assessment

Risk assessment aims to quantify the probability that a specific agent or chemical will give rise to an adverse effect. The principal factors that affect this include the intrinsic properties of the chemical, its use and the exposure levels, and the number and susceptibility of exposed individuals. Consideration of these factors are important because even for highly toxic chemicals if the exposure level is below the threshold for effects, or the number and susceptibility is low, then there may not be a significant risk. However, substances with low toxicity may cause considerable concern if the exposure levels are sufficient to give rise to biologically effective doses in a high number of susceptible individuals. In occupational situations, the number of individuals is usually relatively small and therefore the main determinant is the exposure level. However, for environmental exposures a large number of individuals might receive a biologically effective dose resulting from relatively low exposure levels, and individual susceptibility may represent the main risk determinant.

In the overall procedure of risk assessment, which entails hazard identification, dose–response determination, exposure assessment and risk characterization, biomonitoring is mainly applied in the exposure assessment phase in order to identify exposed individuals and groups and determine their exposure levels. Biomonitoring may also be used in the risk characterization phase to assess health risks for exposed groups depending on exposure levels. Biomarkers of effect can be used as part of health surveillance programmes for early diagnosis of exposure related disease, but the application of biomarkers of effect is usually aimed at determining whether a particular exposure is associated with early effects in the critical organ.

The dose–response assessment is a critical element of the risk assessment process since it establishes the probability or degree of response from different exposure levels. Results of studies with experimental animals are often used with extrapolation to arrive at values for acceptable daily intake and environmental and occupational exposure limits. Where there are uncertainties in making extrapolations across species, large safety factors are often incorporated. To reduce the uncertainties in extrapolations, biomarkers can be used to assess dose, effects and susceptibility in order to derive dose–effect and dose–response relationships directly in humans. In such studies the parent compound or its metabolites in accessible media or target molecules can be used to assess their relationship with

biomarkers of adverse effect, which can be measured as biochemical or physiological abnormalities well before the appearance of disease.

Epidemiological studies are relying increasingly on biomarkers to define exposure to environmental pollution. Biomarkers are, however, surrogate measurements of something that is difficult to measure because, for example, of its inaccessibility. Whilst biomarkers of exposure are expected to increase the precision of traditional approaches to define exposure and outcome that are based on such crude methods as job description and death certificates, the validation of specific biomarkers is still a major challenge.

Biomarkers of effect are perhaps best regarded as indicators of early changes that could later lead to clinical disease (Mutti 1995). When dose–effect and dose–response are known, a specific biomarker of dose may be sufficient to assess the risk of adverse effects. There are situations where biomarkers of dose cannot be used to predict potential adverse effects. In such situations, biomarkers of effect may be useful to understand whether a change in their distribution has occurred due to the chemical exposure. Thus, biomarkers of effect are not proof of disease caused by environmental pollution but tools to understand a process that might eventually lead to adverse effects.

Human risk assessment

For the existing biomarkers of exposure, the most reliable data for human risk assessment is in the field of chemical carcinogenesis. Suitable markers are those provided by protein adducts or urinary metabolites for which there are good methods for quantification and hence a means of determining the exposure dose which is an essential part of the risk assessment. Those markers that provide a more reliable way of quantifying the exposure dose are seen as particularly useful in the risk assessment process. Markers that are closest to the disease should provide the best estimates of risk. In the chemical carcinogenesis research area, mutation and chromosome aberrations are the closest markers for an indication of disease and therefore the detection of mutation is a good indicator of human risk. The correlation of adducts with mutation can therefore provide molecular markers for cancer risk assessment (Watson and Wright 1991, Watson and Van Sittert 1996). The reliability and usefulness of data is both class and compound specific and also related to the nature of the effect. Although chemical carcinogenesis is the most studied and understood, one must be aware that the unique combination of changes leading to cancerous transformation only occur in target cells. Other cells and markers will always be surrogate measures bearing quite a large margin of uncertainty.

There have been relatively few practical applications of biomarkers in assessments to set occupational exposure levels. For human risk assessment, epidemiological evidence is still taken as the reference standard but this is, of course, based on retrospective analyses. Historically, biomarkers have been used to measure mutations in surrogate genes or with cytogenetics to assess overall changes in chromosome structure and number. Although these markers have been shown to be associated with a wide range of carcinogenic exposures they are not on the causal pathway of disease and are therefore not usually true biomarkers of effect. Identification of early causal genetic effects in cancer has led to the development

of biomarkers of early effect in high-risk populations. The biomarkers which measure changes that are frequently observed in cancer patients, include point mutations in genes, e.g. p53, altered gene methylation, aneuploidy and specific chromosome rearrangements such as translocations. The future technologies are anticipated to be able to measure tens of thousands of endpoints from a small blood sample by the use of proteomics and gene array techniques to identify all genetic polymorphisms related to susceptibility. The application of these markers to potentially at risk individuals should result in improved early detection of cancer and better understanding of the risk factors involved. In the occupational situation, there may be considerable ethical issues to deal with as a result of these developments (see below).

Prospects and potential uses for newly developed biomarkers

Toxicogenomics and proteomics

Sequence information on the human genome is now being exploited to detect biological responses to toxic chemicals (Pennie *et al.* 2000, Henry *et al.* 2002, Pennie 2002). This could cause a revolution in toxicology that will affect molecular epidemiology (Tugwood and Beckett 2002). Cells can respond to toxic insults by altering gene expression. This can be measured by the RNA that is transcribed. It is of course important to recognize that not all toxicity is due to changes in gene expression and that such changes may be coincidental rather than causal. Nevertheless, recent developments in high throughput technology permit analyses of large numbers of RNA transcripts so that large numbers of genes can be monitored. The approach receiving most attention, known as microarray or chip technology, uses immobilized cDNA or oligonucleotides on a solid support. Labeled cDNA or RNA probes obtained from test tissues are hybridized to the immobilized target oligonucleotides to permit the monitoring of thousands of genes. The analysis of genes for toxicological studies is known as toxicogenomics. Toxicogenetics, on the other hand, describes the consideration of heritable alterations in the genome that influence the relative susceptibility of an individual to the adverse effects that may arise from exposure to a chemical (Orphanides and Kimber 2003). The emerging area of protein expression, known as proteomics, is based on the detection of alterations in protein expression as indications of perturbations in gene expression. Toxicogenomics and proteomics are complementary methods for monitoring the responses of large numbers of genes following a toxic challenge and could represent the biomarkers of the future.

Of all the newly developed biomarkers for monitoring exposure and assessing risks it is viewed that toxicogenomic and proteomic approaches offer the most promise but the results from these methods also pose considerable difficulties in interpretation (ECETOC 2001). Genomics, transcript profiling (transcriptomics), proteomics and metabonomics are rapidly developing technologies that enable a description of biological events at the level of genetic material (genomics) and its expression. Expression can be studied in the transfer of genetic information (transcriptomics), in the formation of proteins (proteomics), and by determining the metabolites resulting from the proteins (metabonomics). The techniques can

generate massive amounts of data characterizing changes in the presence and amounts of potentially thousands of biomolecules simultaneously. There are therefore tremendous possibilities for research in toxicology. A major issue to overcome in their application is that there is always a response either adaptive or possibly toxic. It is possible to describe altered gene expression provoked by chemicals long before valid interpretations of their meaning can be found. Currently the methods are better suited to investigating and understanding toxicological mechanisms. Care should be exercised in the interpretation of results from these methods because there is the possibility that indiscriminate application of these technologies will lead to the generation of misleading data. The relative lack of reference data could lead to mis- or over-interpretation and subsequently to undue concern by regulatory agencies. There is also a need for the chemical industry as a whole to collaborate with academics and regulators in the development and sharing of such reference data sets. This will help to ensure that the new technologies are appropriately applied and that agreement is reached on the interpretation of the data that they generate, with due consideration to the transient nature of gene expression, most often representing adaptive responses not necessarily leading to pathological processes.

A wide variety of cDNA microarrays are available for studying gene expression, which so far have found only limited applications in biomarker research. The main reasons for this are firstly the cost and secondly the requirement for relatively large amounts of high quality RNA. mRNA is an unstable material and is rarely collected and stored appropriately in molecular epidemiology studies. Protein arrays and proteomics have great potential in biomarker research as they may identify a pattern of protein expression associated with a particular exposure or early onset of disease. Large banks of biological materials are already available for analysis by these assays. Some examples have indicated that proteomics approaches are more sensitive than conventional toxicological assays, detecting altered protein expression at lower doses and earlier time points. An exciting new development expected to be applied in the future is based on nanoparticles designed like a nanoscale barcode (Nanobarcode) labeled with antibodies such that thousands of proteins can be recognized simultaneously.

Mass spectrometry

Mass spectrometry is a universal chemically specific detector and therefore is an ideal method for applications in biomarker monitoring. With the continuing improvement in sensitivity of detection, it also offers much promise in its use for detecting and characterizing biomarkers especially in the area of 'backgrounds' in individuals who are not knowingly exposed to chemicals. LC/MS/MS for adducts and for metabolites in urine offers definitive identification of exposure.

Need for development of analytical methodology

Analytical methodology for existing biomarkers is sufficiently well developed to permit routine human monitoring for only a relatively small number of chemicals. These are mainly adduct or metabolite measurements for chemicals such as

ethylene oxide, propylene oxide, benzene, styrene, and acrylamide. Immunochemical methods based on recognition of adducts by antibodies require more development but could become useful in routine screening because the conventional analytical procedures are often too labour intensive for routine application. Of the general methods for monitoring chemical exposures, cytogenetic methods such as measurement of chromosome aberrations or micronuclei are most well established and are already in routine application. It is anticipated that further development of these cytogenetic methods is not required. The methods employed in toxicogenomics, proteomics and metabonomics require considerable development and whilst there have been some examples of genetic screening it is anticipated that development is necessary before applications in human monitoring will be possible on a routine basis.

Validations of biomarker assays/tests

Validation studies for some of the biomarker assays have been carried out in the form of inter-laboratory trials and ring tests. Cytogenetic assays have been generally well validated and results between laboratories compare well in general. The IARC has been instrumental in the organizing and administration of ring tests for DNA adduct determinations. Typically these involve about 15 laboratories carrying out the experimental studies, followed by workshops to present and discuss the findings, and then compilation and publication of the results (Phillips and Castegnaro 1993, Phillips and Castegnaro 1999). A major concern for DNA adduct measurements, based mainly on ^{32}P-post-labelling or immunochemical methods, revealed by these ring tests has been the large variation in results from different laboratories analysing samples from the same or similar exposures.

There have been limited trial studies comparing haemoglobin adducts measured by different laboratories, as there are fewer laboratories with the skill and equipment to execute these analyses. Nevertheless the GC/MS methods used for haemoglobin analyses are considered quantitatively very reliable and may not suffer the wide discrepancies between laboratories seen for DNA adduct measurements. There have been limited comparisons between laboratories for haemoglobin adduct measurements and in some instances variations have been noted (Törnqvist *et al.* 1992). Because haemoglobin adducts are viewed as a very good indicator of exposure to reactive electrophilic chemicals, more comparative inter-laboratory studies would be valuable to assist in the validation of their use. It would also be of benefit if more individual validation studies were carried out. The studies should be carried out in conjunction with extensive ambient monitoring over timescales consistent with the time constant of the biomarker studies. More chemical reference standards are required for calibrations and validations of the methods and these are often difficult to obtain unless chemical expertise is incorporated into the investigations. The monitoring of occupational and environmental exposures to mixtures is particularly difficult especially in the definition of appropriate standards and the choice of marker.

Quality assurance procedures

There is a general concern about whether the quality assurance procedures in place in reported studies are adequate and what procedures should be in place for human studies. Most of the studies involving human biomonitoring have been research studies and it has been rather uncommon to incorporate a quality assurance element into the monitoring programmes. There have been some exceptions. In the transitional epidemiological study of workers exposed to 1,3-butadiene reported by Albertini *et al.* (2003) which integrated comprehensive exposure assessments with a series of biomarker analyses there was an independent audit and review of the results from the participating laboratories (Kensler *et al.* 2003). Rather rarely in this field of studies do the quality assurance procedures match clinical needs. Although researchers carry out studies using good research practice, due to the nature of investigative research relatively few studies could be accredited to GLP (good laboratory practice) standard. In general there is a need for an improvement in the quality assurance procedures in human studies. The consistent and more widespread use of proper chemical standards and inter-laboratory comparisons would improve the confidence in results. The cost of studies would have to increase substantially to meet these additional requirements.

Ethical issues arising from human biomonitoring

In general the ethical issues arising from obtaining blood or body fluid samples for analysis are straightforwardly managed by consultation with the subjects and good study design and ethical review, since, in the main, the methods are non-invasive. It is hoped that molecular and genetic biomarkers will assist in the identification of which exposures are present in a given environment and which of these exposures play important roles in the development of disease. It has been suggested that the application of new molecular and genetic markers will result in increased scrutiny of biomarker research with the necessity of consideration of the possible social consequences of the studies (Sharp and Zigas 2002). There is a possibility that molecular biomarkers of exposure may be considered by laypersons to be more significant than other sources of information on exposure risk. Biomarkers of clinical effect might be used in establishing claims for an increased risk of future disease due to the toxic exposure. Markers of genetic susceptibility could identify individuals at elevated risk and this could have consequences for their suitability for employment in particular work operations.

Normally the data from an individual does not provoke concern until it is compared with others in the data set when individuals with high outlying values may be revealed. Confidentiality appears to be the major aspect of concern in respect of ethical issues arising from human monitoring studies and the handling of the generated data, especially with regard to information relating to an individual's DNA. Samples for testing should therefore always be coded for security of information. In biomarker studies aimed at measuring DNA adducts, the adduct levels arising from a given exposure provide a definition of an individual's susceptibility. As a consequence there is always a difficult choice about what information to tell individuals who have been tested. It is not certain whether the

exposures for those individuals with high adduct levels, for example, have been higher or whether their genotype makes them more susceptible than others in the group. For occupational hygiene situations such results can be used to identify whether exposure standards need to be improved, or possibly, whether the working practices of the individuals with outlying high results are not satisfactory, such as by the misuse or lack of use of protective equipment/measures.

Important future directions and prospects for biomarker research

An important objective for the future direction of biomarker studies is to improve and apply biomarkers to investigate individual differences in rates of uptake, elimination, metabolism and repair of toxic chemicals and their products. There is a need for more specific markers to establish relationships with exposure. Moreover, it is important to establish whether there are thresholds of effects and determine whether there are levels of exposure with no observable effects. The development of standardized methods, which have been properly evaluated and validated, especially in inter-laboratory trials, are also considered important. Mass spectrometry is a universal method of detection and therefore it is considered important to develop this further for biomonitoring and biomarker studies. Mass spectrometry can be used for screening for common molecular fragments. The technique of laser desorption ionization (MALDI) is being effectively employed in proteomics for characterization of proteins. Mass spectrometry is already very useful for quantifying biomarkers of exposure but better biomarkers of effect are required and proteomics should be particularly useful. Adduct reference standards are always necessary and a higher level of chemical input is necessary for many research programmes. The development of toxicogenomics, proteomics and metabonomics techniques is viewed as very important, especially for studies of mechanistic aspects. There is considerable excitement amongst researchers in these fields because of the potential prospects, which include the potential for human health benefits in drug development. It is, however, likely that for some time to come these methods will not be able to measure effects at the low doses of chemicals humans are exposed to, and, it is uncertain whether and when this objective will be achievable. Although the techniques are evolving rapidly better analyses are still required and better definitions of which genes to analyse are necessary. Procedures for analysing increments in the levels of existing proteins are required and this is expected to be very difficult.

Biomonitoring of carcinogen exposure

The use of molecular biomarkers for the biomonitoring of exposures to carcinogens has been a significant driving force in the development of biomarkers and therefore warrants a more detailed discussion. The concept of biomonitoring of carcinogen exposure through the measurement of the products (adducts) of the interactions of carcinogens with nucleic acids (target molecules) and proteins, as noted above, first originated more than 27 years ago in the Ehrenberg group in Stockholm (Ehrenberg *et al.* 1977). The application of biomarkers in occupational

toxicology and epidemiology was a significant turning point for these disciplines. The first major interest was in the biomonitoring of exposure to carcinogens, causing a move from estimates of external exposure to internal measures of dose, then to markers of target dose. Efforts to measure the effects of carcinogens at the molecular level have occupied a very substantial proportion of the research work on biomarkers. As noted previously, the macromolecules that have received most attention and study in respect of adduct formation are DNA, haemoglobin and albumin. DNA damage was already known to be associated with mutation and cancer and so was an obvious target molecule on the basis that the extent of adduct formation would bear a relationship to the subsequent stages of mutation in the carcinogenic process (figure 4). DNA adducts are also viewed as biomarkers in the prevention of chronic degenerative, e.g. cardiovascular, diseases, either by avoiding exposure to adduct forming agents or by chemo preventative means or dietary modification (De Flora *et al.* 1996).

Genotoxic electrophilic chemicals also react with nucleophilic centres (nitrogen, oxygen, sulphur) in blood proteins such as haemoglobin or serum albumin (figure 4). There are significant advantages in using haemoglobin adducts rather than DNA for monitoring human exposures and risk assessment (Törnqvist and Ehrenberg 1994, Törnqvist and Hindso Landin 1995, Törnqvist *et al.* 2002). These advantages include the ease and accessibility of obtaining relatively large amounts of haemoglobin and the greater potential for genotoxic chemicals to react with proteins compared with DNA. In addition, human red blood cells have a long lifetime of about 120 days, there is no repair of the adducts formed with blood proteins, and, the adducts are usually stable. Human monitoring using blood proteins can therefore be carried out several months after the suspected exposure. In contrast, DNA from susceptible human tissues is not readily accessible in large quantities. DNA adducts are also repaired and at different rates depending on the tissue, cell type and DNA region. For the relatively few compounds studied there appears to be proportionality between the formation of haemoglobin adducts and DNA adducts especially at low doses of carcinogen exposure (e.g. for ethylene oxide, Potter *et al.* 1989; 1,3-butadiene, Booth *et al.* 2004a,b).

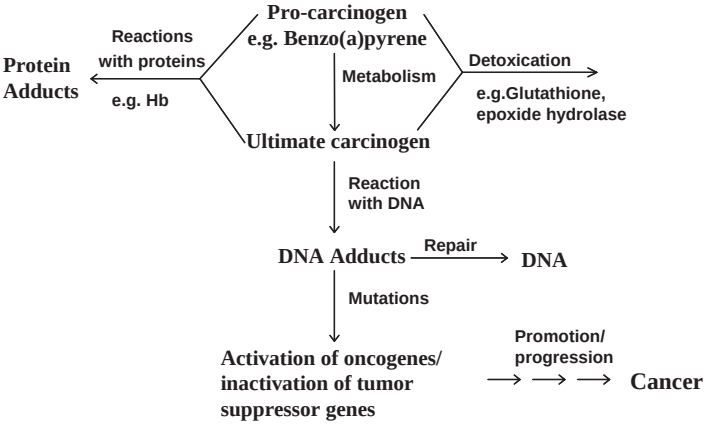

Figure 4. Mechanism of chemical carcinogenesis (Watson *et al.* 1994).

Developments in analytical methods for monitoring of carcinogen exposures

A wide range of analytical techniques has been developed for detecting and quantifying carcinogen adducts with macromolecules. For protein adducts these include: immunoassays, gas chromatography mass spectrometry (GC-MS), HPLC, and for DNA adducts: immunoassays, ^{32}P-post-labelling, HPLC, fluorescence, mass spectrometry. These methods are all highly sensitive and have exceptionally low levels of detection. As previously noted, a consequence of this has been the significant discovery that adducts of many carcinogens have been found to be present in proteins and DNA from supposedly unexposed individuals and populations. The origins of these 'background' levels in DNA and blood proteins were, until relatively recently, largely unknown. In some instances, such as the cases of acrylamide and heterocyclic amines, the exposures have been shown to arise through intake from cooked foods in the diet (Totsuka *et al.* 1996, Wakabayashi *et al.* 1997, Tareke *et al.* 2002).

Detection of adduct formation in proteins

Ehrenberg's group in Stockholm was the first to develop and use methods based on haemoglobin adducts for detecting, identifying and quantifying exposures to genotoxic carcinogens. This pioneering work was essentially the foundation for future studies on biomonitoring of genotoxic carcinogens. A main aim of the work was to improve the risk assessments for human exposure to genotoxic chemicals. A number of analytical methods have since been developed for measuring protein adducts, the majority of these relying on mass spectrometry detection (reviewed in Farmer 1994, 1995, 1999). The most widely applied and most successful procedure involves specific cleavage of adducts at the valine termini of haemoglobin by pentafluorophenyl isothiocyanate in a modified Edman degradation and subsequent analysis by GC-MS (Törnqvist *et al.* 1986) (figure 5). This procedure

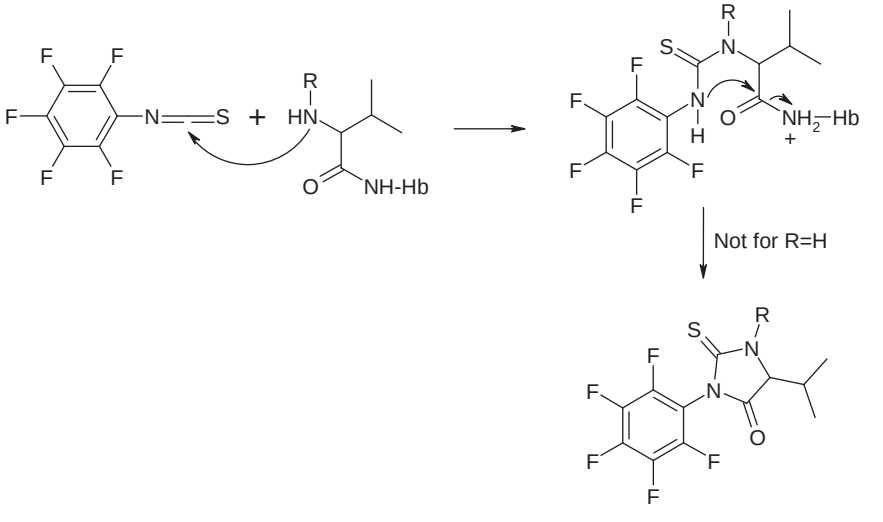

Figure 5. Modified *N*-alkyl Edman degradation for haemoglobin adduct analysis.

has been extensively developed and there have been many applications in different laboratories (Boogaard 2002, Törnqvist *et al.* 2002). Mass spectrometric detection has played a very important role in achieving the high sensitivity of detection necessary for haemoglobin adduct detection and characterization. Whilst immunochemical methods were also developed early on and were used for the determination of haemoglobin adducts (Lee and Santella 1988, Wraith *et al.* 1988) the majority of applications have been for measurement of exposure to polycyclic aromatic hydrocarbons.

Detection of adduct formation in tissue DNA

Adducts in human tissue DNA resulting from occupational and environmental exposures occur at extremely low levels and the detection of these adducts has therefore required the development of highly sensitive analytical techniques for their measurement and identification. The ^{32}P-post-labelling analysis technique developed originally by Randerath *et al.* (1981) is one of the most widely applied methods for detecting DNA adducts. The original procedures (reviewed in Watson 1987, Beach and Gupta 1992) involve the incorporation of radioactivity, *via* polynucleotide kinase catalyzed transfer of the γ-phosphate of ^{32}P-labeled ATP, specifically into non-radioactive DNA adducts. Following isolation of DNA, it is enzymically hydrolyzed to deoxyribonucleoside 3′-monophosphates. In the original procedure the mixture of normal nucleotides and adducted nucleotides was enzymatically phosphorylated with high specific activity ^{32}P-ATP to give mixtures of 3′,5′-bisphosphates which were separated on multidimensional ion exchange thin layer chromatography. Resolved adducts were quantified by cutting out the radioactive spots and radioassay by Cerenkov counting. A major advance was the development of methods for the enrichment of adducts from the normal nucleotides before the ^{32}P-labeling step. The methods now most widely used are butanol extraction (Gupta 1985), nuclease P1 digestion of normal nucleotides (Reddy and Randerath 1986) and immunaffinity chromatography (Booth *et al.* 1994). The limit of detection for adducts from polycyclic aromatic hydrocarbons is about one adduct per 10^9–10^{10} normal nucleotides using μg quantities of DNA. This is equivalent to about 1 adduct per cell. The ^{32}P-post-labelling technique has had many applications (Phillips 1997).

Immunochemical methods have also been widely used for the determination of DNA adducts. The chemical structures of adducts may influence the methods chosen for detecting DNA adducts, e.g. fluorescence techniques can be used for some polycyclic aromatic compounds (reviewed in Farmer 1994, 1995). Mass spectrometry adds a high degree of specificity to a quantitative method, especially when used in combination with isotope dilution mass spectrometry and selected reaction monitoring. The main disadvantage of mass spectrometry for DNA adduct quantification is that it is not as sensitive as the most sensitive techniques such as ^{32}P-post-radiolabeling (Koc and Swenberg 2002). The difference in sensitivity is, however, being reduced as the technology improves particularly with gas chromatography-electron capture high-resolution mass spectrometry (GC-EC-HRMS) and liquid chromatography electrospray ionization mass spectrometry

(LC-ESI-MS-MS). Detection limits in the low attomole range are possible with GC-EC-MS and low femtomole levels with LC-ESI-MS-MS methods.

Data relating human exposure with biologically effective dose (amount of chemical bound to DNA) have been obtained for a variety of chemical carcinogens, including polycyclic aromatic hydrocarbons, aromatic amines, heterocyclic amines, aflatoxins, nitrosamines, cancer chemotherapeutic agents, styrene, and malondialdehyde. However, because of the quality of the exposure documentation, dosimetry has not been precise with most environmental and occupational exposures, even though increases in DNA adduct levels in human blood cells correlate approximately with dose. In general lowering of exposures resulted in decreasing DNA adduct levels. Future advances will be dependent on proper characterization of DNA adducts formed in human tissues, better precision in the dosimetry and correlation of DNA adducts with cancer risk (Poirier 1997).

Cytogenetic markers as cancer biomarkers

With the advancement of techniques in cytogenetics, extensive studies have been carried out with markers such as SCEs, MNs and other changes that are viewed to represent genomic damage. These types of endpoints are unspecific for application for identifying new hazards where the human carcinogenic potential is uncertain. The four most frequently used cytogenetic endpoints used in hazard identification are CAs, MNs, aneuploidy and SCEs. The main reason for evaluating structural chromosome aberrations is that there is a clear association between chromosome rearrangements and cancer (Solomon *et al.* 1991, Mitelman 1994). The most suitable cells for analysis are peripheral blood lymphocytes or bone marrow cells.

Examples of monitoring of occupationally exposed workers

Many studies have been carried out to monitor adduct formation in workers occupationally exposed to complex mixtures of chemicals, e.g. polycyclic aromatic hydrocarbons. Occupations studied have included foundry workers (Phillips *et al.* 1988b, Savela *et al.* 1989, Reddy *et al.* 1991) coke-oven workers (Hemminki *et al.* 1990, Øvrebo *et al.* 1992), roofers and pavers (Herbert *et al.* 1990), fire-fighters (Darcey *et al.* 1992), miners (Qu *et al.* 1997), bus drivers (Hemminki *et al.* 1994b) and garage workers (Schoket *et al.* 1999). Sometimes relatively large groups of workers have been investigated so that the statistical significance of small increments in adduct levels could be determined. In most studies, smokers were identified so that the contribution from smoking could be established. Many of the studies have shown differences in response between individuals and, as noted earlier, this is now recognized as possibly being due to inherent genetic factors. Some of the studies have shown a difference in adduct levels between exposed workers and controls. Workers with consistent high exposure, e.g. coke-oven workers showed significant increments in adducts above controls. However, the work related exposure of American fire-fighters in Kuwait after the first Gulf War did not give rise to a significant increase in adducts in the majority of the workers (Darcey *et al.* 1992). In

a review of monitoring occupational and environmental exposures to polycyclic aromatic compounds (Brandt and Watson 2003) it was found that urinary 1-hydroxypyrene was a suitable marker for internal exposure to polycyclic aromatic hydrocarbons. In contrast DNA adducts mostly determined in white blood cells did not show good correlations with exposure to polycyclic aromatic hydrocarbons in a variety of workplace and exposure situations. This was in contrast to the findings from controlled laboratory studies where there were correlations between exposure to polycyclic aromatic hydrocarbons and the levels of adducts in tissues. A possible explanation for the lack of correlation between DNA adducts and occupational exposures to polycyclic aromatic hydrocarbons is that unquantified sources of exposure, such as from the diet or in the cooking of food, could make a large contribution to the total human exposure load compared with the quantified occupational sources. MNs and SCEs were also found to be unsatisfactory as biomarkers for exposures to polycyclic aromatic compounds. From a rather limited amount of data, chromosome aberrations did show a correlation with exposure to polycyclic aromatic hydrocarbons. Chromosome aberrations are recognized as having an association with cancer and therefore may be indicators of increased cancer risk from exposure to polycyclic aromatic compounds.

Monitoring exposure to specific individual chemicals

Biomarkers have been used successfully for many years to monitor occupational exposure to specific individual chemicals, e.g. ethylene oxide (Calleman *et al.* 1978, Van Sittert 1984, Van Sittert *et al.* 1985, Wraith *et al.* 1988). Workers in butadiene manufacturing and the butadiene styrene rubber industry have also been extensively studied (IARC 1999a). More recently a range of biomarkers in blood and urine have been evaluated for their sensitivities as indicators of low-level exposure to 1,3-butadiene and use in risk assessment (Van Sittert *et al.* 2000, Albertini *et al.* 2001, Boogaard *et al.* 2001). The biomarkers evaluated were metabolic genotypes, urinary metabolites, haemoglobin adducts, HPRT mutations, SCEs and CAs. Urinary metabolites and haemoglobin adducts correlated with exposure levels but there was no significant correlation with HPRT mutations or the cytogenetic endpoints (Albertini *et al.* 2003). For example, in this audited and reviewed (Kensler *et al.* 2003) inter-laboratory transitional epidemiology study on occupational exposures to butadiene it was found that valine adducts formed in haemoglobin by the butadiene metabolites, 1,2-epoxybut-3-ene and 3,4-epoxybutan-1,2-diol, and also the urinary metabolites 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane, 1-hydroxy-2-(*N*-acetylcysteinyl)but-3-ene and 2-hydroxy-1-(*N*-acetylcysteinyl)but-3-ene were significantly correlated with group and individual mean exposure levels of butadiene (Albertini *et al.* 2003). Both the urinary metabolite 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane and the valine adduct from 3,4-epoxybutan-1,2-diol were detected as a background in controls, an observation made previously, not surprisingly with smokers, but also in a range of unexposed animals (Swenberg, *et al.* 2000). As already noted it is clear that there is a need to establish the source and significance of such backgrounds from both a scientific and regulatory standpoint. Data from the haemoglobin adduct

measurements from one of the laboratories in the study of Albertini *et al.* (2003) indicated that there was a ten-fold range for inter-individual differences in the human metabolism of butadiene (Swenberg *et al.* 2001). For ethylene oxide and propylene oxide, correlations were found between airborne exposure levels of male operatives working on a petrochemical plant and haemoglobin adducts in blood measured by the modified N-alkyl Edman method (Boogaard *et al.* 1999). In a small scale pilot study, correlations were found between DNA and haemoglobin adducts and SCEs in workers from a Chinese propylene oxide producing plant but it was not certain whether the increases in SCEs were from other confounding sources (Czene *et al.* 2002).

Environmental exposures and smoking

Smoking represents a predominant source of non-occupational exposure to chemicals, though diet can also contribute significantly. The oral intake of polycyclic aromatic hydrocarbons from the diet may reach a level similar to those after occupational exposure (Buckley and Lioy 1992). The cooking of food also can give rise to significant exposures to carcinogens. The sum of the eight carcinogenic polycyclic aromatic hydrocarbons found in fumes caused by frying meat were greater than the levels on coke-oven plants (Chen and Chen 2003). It has been reported that there is a correlation between DNA adduct levels in human lung and cigarette smoking (Phillips *et al.* 1988a). Another study showed slightly increased levels of DNA adducts in cervical epithelium in women smokers compared to non-smokers (Simons *et al.* 1993). In a study of tissues from human autopsy samples a strong association was found between levels of adducts in lung DNA and smoking (Routledge *et al.* 1992). However, there was much weaker association between smoking and increased levels of adducts in bladder DNA. It has been shown that levels of aromatic DNA adducts in white blood cells correlated with the degree of smoking but saturation occurred at high exposures (Van Schooten *et al.* 1997). Levels of aromatic adducts in lymphocytes of smokers became saturated at around 15 cigarettes per day (Dallinga *et al.* 1998) and the suggested saturation levels were considered similar to those that could be caused by a high dietary intake of polycyclic aromatic hydrocarbons (Hemminki *et al.* 1997). There was no difference between the adduct levels in lymphocytes of newspaper vendors in Milan working in high or low traffic density areas (Yang *et al.* 1996). The heavy smokers analysed had significantly higher levels of adducts than the non-smokers. Inter-individual variability in the polycyclic aromatic hydrocarbon-DNA adduct responses to smoking have been noted (Dickey *et al.* 1997). No difference was observed between DNA adduct levels in lymphocytes of bus drivers in Stockholm compared with controls (Hemminki *et al.* 1994b). However, a study on personnel who serviced and loaded diesel vehicles did find a significantly higher level of adducts in the lymphocytes in the exposed group (Hemminki *et al.* 1994a). In a study by Lewtas *et al.* (1997) higher levels of adducts were found in white blood cells of environmental control subjects than in those of coke-oven workers. Whilst DNA adducts have been used mainly as biomarkers of exposure to genotoxic agents there

is epidemiological evidence that some DNA adducts can be predictive of cancer risk, e.g. aflatoxin B1 and hepatocellular carcinoma (Qian *et al.* 1994).

Urinary metabolites as biomarkers of exposure to carcinogens

Measurement of the excretion of urinary metabolites, e.g. 1-hydroxypyrene for measurement of exposure to polycyclic aromatic hydrocarbons is a widely applied procedure. Methods for determination of 1-hydroxypyrene have been extensively validated in a broad range of industrial settings with potential exposure to polycyclic aromatic hydrocarbons and in control populations (Boogaard and Van Sittert 1994). The method has been used for identifying sources and routes of exposure (Boogaard and Van Sittert 1995b, reviewed in Dor *et al.* 1999). In a review of many types of occupational exposures to polycyclic aromatic hydrocarbons excreted 1-hydroxypyrene was found to correlate well with exposures to these compounds (Brandt and Watson 2003). Mercapturic acids in urine have also been used for monitoring exposure to a number of specific chemicals, e.g. epichlorohydrin and styrene (for reviews, see Van Welie *et al.* 1992, and De Rooij *et al.* 1998). For the monitoring of low-level benzene exposures, *S*-phenyl mercapturic acid in urine was found to be a superior biomarker compared with *trans*, *trans*-muconic acid or phenol (Boogaard and Van Sittert 1995a, 1996). For styrene, inter-individual differences were found in urinary excretion of the end-products, mandelic acid and phenylglyoxylic acid as well as the phenylhydroxyethyl mercapturic acids. It was found that the glutathione transferase M1 (GST M1) genotype was the most significant parameter affecting the phenylhydroxyethyl mercapturic acid excretion and when this was taken into account the mercapturates were a good biomarker for biomonitoring (De Palma *et al.* 2001, Haufroid *et al.* 2002).

Cancer risk estimation for human exposures to chemicals

At this time there is still no fully validated and generally applicable method for relating macromolecular adducts with risk, although adducts can clearly define the internal or target dose. Ehrenberg proposed that if the *in vivo* dose of a carcinogen at its critical target (target dose) could be measured then cancer risks could be estimated by comparison with an agent for which the human cancer risks are known. γ-Radiation was proposed as a reference standard because it is the environmental factor for which the relationship between exposure dose and risk in humans is best known. The Stockholm group have determined 'radiation equivalent values' for the induction of mutations caused by a number of direct acting monofunctional alkylating agents using a wide range of genetic end-points in biological systems including bacteria, plants and mammalian systems (Ehrenberg 1979, 1988, Wright *et al.* 1988). The radiation equivalent value for a given alkylating agent was found to be similar in each of the test systems. Experimentally determined radiation equivalent values can be used to convert target (haemoglobin) doses of genotoxic chemicals in human individuals and populations exposed to low levels of chemicals into equivalent doses of radiation (Watson and Van Sittert 1996). Using the target dose approach together with a multiplicative model rather than an additive model

indicated that the relative risk coefficients for genotoxic chemicals were independent of species and that relative cancer risks determined in animal tests also apply to humans (Granath *et al.* 1999). Of the rather small number of chemicals investigated, ethylene oxide is the most extensively studied model.

Concluding remarks

Developments in analytical chemistry have considerably improved the capability to characterize individual human exposure to occupational and environmental contaminants by allowing their measurement in accessible biological media at the nanomolar level. Biomarkers of individual susceptibility are being investigated in both cancer and non-cancer epidemiology, particularly as complements for risk assessment. For genotoxic compounds protein adducts such as those formed with haemoglobin appear to be an especially effective way of determining individual exposure doses. For other organic chemicals, excreted urinary metabolites can give a useful indication of exposure. It is acknowledged that host dependent factors may not only play a key role in such multifactorial diseases, but also affect the low dose region of the dose–response relationship for toxic outcomes.

A very exciting and promising area of research is the development and validation of biomarkers of early effect, notably in the area of genomics and proteomics. The use of more quantitative and sensitive endpoints is anticipated to increase the capability to identify toxic responses to chemicals in the workplace and the general environment. These advances are likely to result in a better characterization of dose–response relationships especially in the low dose exposure region, and this is expected to be an important step in establishing the risks involved and, if necessary, steps to implement reductions in exposure. The building of reliable databases of biomarkers offers the promise of integrating information from genome research programmes which may find application in the prediction of health risks and the prevention of environmental and occupational diseases. The use of these genetic biomarkers may raise important ethical considerations.

In the case of risk assessment there is a need to adopt rational approaches that do not suffer from the shortcomings of conventional epidemiology and long-term animal studies. Molecular approaches based on mechanistic insights of the toxicological processes offer some promise for a way to overcome these drawbacks. The advent of highly sensitive quantitative biomonitoring methods for measuring adducts directly in humans provide a means for dose determination and therefore the basis for risk assessments at the low exposure levels encountered in the workplace and general environment.

Acknowledgements

Financial support (W. P. W.) was provided by the Human Exposure and Tiered Risk Assessment (HETRA) Group of the Long Range Research Initiative (LRI) of the European Chemicals Council (CEFIC), Brussels, Belgium. The following persons are especially thanked for useful discussions and opinion forming exercises during the preparation of this review: Dr R. Baan, IARC, Lyon; Professor H. Bartsch, DKFZ, Heidelberg, Germany; Professor P. B. Farmer, University of

Leicester, UK; Professor E. M. Faustman, University of Washington, USA; Professor D. H. Phillips, Institute of Cancer Research, London, UK; Professors S. M. Rappaport and J. Swenberg, University of North Carolina, USA; Dr D. Segerbäck, Karolinska Institute, Stockholm, Sweden; and Professor M. Törnqvist, University of Stockholm, Sweden. The statements and opinions expressed are nevertheless attributable solely to the authors.

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