Biomarkers: A tool for monitoring pesticide pollution

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ABSTRACT

The presence of xenobiotics in the environment always represents a risk for living organisms. Thus, there is a need to detect toxicity in the organism, and the concept of intoxication is related to specific organ alterations and clinical symptoms. Moreover, the relationship between the toxic levels within the organism and the toxic response is rather complex and has a difficult forecast because it depends on several factors, namely toxicokinetic and genetic factors. One of the methods to quantify the interaction with xenobiotics and its potential impact on living organisms, including the human being, is monitoring by the use of the so-called biomarkers. Biomarkers are used to detect the effects of pesticides before adverse clinical health effects occur. Pesticides and their metabolites are measured in biological samples, serum, fat, urine, blood, or breast milk by the usual analytical techniques. Biochemical responses to environmental chemicals provide a measure of toxic effect. A widely used biochemical biomarker, cholinesterase depression, measures exposure to organophosphorus and carbamate insecticides. Techniques that measure DNA damage (e.g., detection of DNA, protein and haemoglobin adducts) provide a powerful tool in measuring environmental effects. Determination of cytogenetic markers help in of monitoring populations occupationally or environmentally exposed to known or suspected mutagenic-carcinogenic agents. Thus suitable bioindicators, containing specific biomarkers hold the future trend of environmental monitoring.

Keywords: Biomarkers; human and environmental toxicity; classification; Pesticide pollution; Biochemical and molecular markers as a tool.

The National Academy of Sciences (1996) defines a biomarker or biological marker as a xenobiotically induced alteration in cellular or biochemical components or processes, structures or functions that is measurable in a biological system or sample. Silbergeld *et al.*, (1994) defines biological markers as physiological signals that reflect exposure, early cellular response or inherent or acquired susceptibilities, which provide a new strategy for resolving some toxicological problems. *Sensu stricto*, we define a biomarker as a biological response to a chemical or a group of chemical agents but not the presence of the agent or its metabolites within the body (internal dose). However, there is no doubt that the measurement of a xenobiotic in a biological system or sample is a bioindicator of exposure,

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and thus it could be considered a biomarker. Biological monitoring has advantages over environmental monitoring because it measures the internal dose of a compound. Inter-individual differences in absorption, bioavailability, excretion and DNA repair should be taken into account. Moreover, intra-individual differences, as a consequence of particular physiopathological alterations occurring in a specific period of time, also should be considered. This involves an individualized biological control to evaluate the exposure to a particular xenobiotic. The organism acts as an integrator of exposure and several organism acts as an integrator of exposure and several physiological factors, which modulate the uptake of toxic. Thus, we may state that a collective cannot be assimilated as a homogeneous group of individuals exposed to a xenobiotic of physicochemical properties under reproducible and standard conditions. The use of biological markers in the evaluation of disease risk has increased markedly in the last decade. Biomarkers are observable end points that indicate events in the processes leading to disease. They are particularly useful in the evaluation of progressive diseases that manifest their symptoms long after exposure to initiating factors. In such cases, traditional early warning symptoms of developing disease may be lacking. At the same time, the disease, once clinically apparent, may be essentially irreversible. The two main research fields in the use of biomarkers in toxicology are environmental toxicology and industrial toxicology, the latter being one of the most relevant and important branches of medical toxicology.

Definitions

The term "biomarker" is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological. However, discussion in this monograph is limited to chemical agents. Three classes of biomarkers are identified:

- ❒ Biomarker of exposure: an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism;
- ❒ Biomarker of effect: a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the

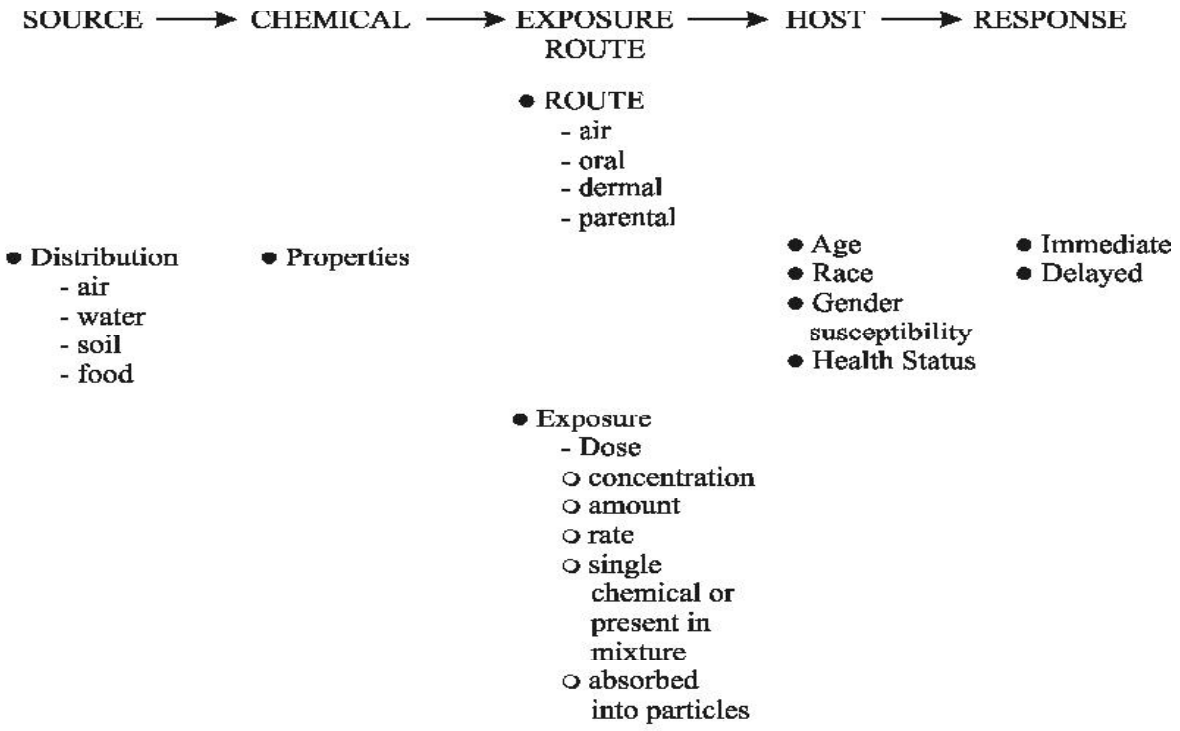

Fig. 1 : Some Critical factors influencing interaction between host and chemical

magnitude, can be recognized as associated with an established or possible health impairment or disease;

- Biomarker of susceptibility: an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance.

Selection and validation of biomarkers

The process of selection and validation requires careful consideration of the specificity and sensitivity of the biomarker as a measure of the contribution of the exposure to an observed adverse health outcome. A similar process must be applied also to establishing the accuracy, precision and quality assurance of the analytical procedure for measurement of the selected biomarker. Before discussing the criteria for the selection and validation of biomarkers of exposure, effect and susceptibility, and their application to the risk assessment process, it is necessary to consider key factors that can influence the host reaction to xenobiotic chemicals. Fig. 1 summarizes some of the various factors that influence the interaction between host and chemical. Many factors require consideration in the process for selection and validation of a biomarker. To select the most appropriate biomarker requires several steps:

(1) the identification and definition of the end-point of interest;

(2) the assembly of the data base to document the relationship between the chemical exposure, the possible biomarkers and the end-point. This will include data from in vitro, mammalian and human studies, with assessment of the validity of data and the study protocols;

(3) selection of biomarker(s) specific to the outcome of interest with careful consideration of the biomarker to identify what is being quantified, to assess the sensitivity and specificity of the marker in relation to exposure, and the significance with respect to health outcome or pathological change over time;

(4) consideration of specimens potentially available for analysis, with emphasis on protecting the integrity of the specimen between collection and analysis, and a preference for non-invasive techniques;

(5) review of the analytical procedures available for quantification of biomarkers and their limitations with respect to detection limit, sensitivity, precision and accuracy;

(6) establishment of an appropriate analytical protocol with provision for quality assurance and quality control;

(7) evaluation of intra- and inter-individual variation for a non-exposed population;

(8) analysis of the data base to establish dose-effect and dose-response relationships and their variation, with special emphasis on susceptible individuals;

(9) calculation or prediction of risk to human health either for the general population or a sub-group; and

(10) review of ethical and social considerations.

Biomarkers of exposure

These allow measurement of the internal dose by chemical analysis of the toxic compound or metabolite in body fluids or excreta such as blood, urine and exhaled air Internal dose also may mean the amount of a chemical stored in one or several body compartments or in the whole body. This usually applies to cumulative toxic chemicals. For example, the concentration of polychlorinated biphenyl (PCB) in blood is a reflection of the amount accumulated in the main sites of deposition (i.e. fatty tissues). The internal dose reflects the amount of chemical bound to the critical sites of action. Bernard and Lauwerys classified the biomarkers of exposure into two subgroups–selective and non-selective-according to their selectivity test, which is based on the direct measurement of unchanged chemicals or their metabolites in biological media. The non-selective tests are used as non-specific indicators of exposure to a group of chemicals. As examples of non-selective exposure tests, the determination of diazo-positive metabolites in urine for monitoring exposure to aromatic amines, the analysis of thioethers in urine and the determination of the mutagenic activity of urine can be cited. When assessing the usefulness of a particular exposure biomarker, one must consider two

aspects of validity: analytical and toxicokinetic. If a person has had only a single, recent exposure to a chemical, the level of biomarkers with short half-life will be high relative to those with a longer half-life. With continuing exposure, the levels of markers with both shorter and longer half-lives should be high. Henderson *et al.,* (1989) showed that if a person was exposed in the more distant, rather than the more recent, past, only the biomarkers with the longer half-lives will be detectable (Fig. 2.). Thus by analysing several biomarkers with different half-lives (e.g., haemoglobin adducts in the blood, metabolites of the chemical in urine, parent compound in blood) from a single individual at a single point in time, more information may be obtained about the nature of the past exposure than from use of a single biomarker. The relative abandance of biomarkers have inverse relationship with their amount formed. Thus half-life of different biomarkers in their decreasing order is urinary adduct > haemoglobin adduct > DNA adduct > Albumin adduct > urinary metabolites, whereas the order of relative abandance is urinary metabolites > albumin adduct > haemoglobin adduct > urinary adduct > DNA adduct. For optimal analytical quality standardization is needed, but the specific requirements vary considerably between individual toxicants. Major areas of concern include: preparation of the individual, sampling procedure and sampling handling; and the measurement procedure, which encompasses technical factors such as calibration and quality assurance procedures. Life events, such as reproduction and senescence, also may affect the toxicokinetic of a xenobiotic.

Biomarkers for pesticides

Organochlorine (OC) compounds are a broad class of pesticides that were widely used as insecticides in the 1950s and 1960s. In subsequent years, their use was discontinued in many countries because of a persistent contamination of the environment. Thanks to studies on plant workers and human volunteers with long-term exposure, biological half-lives are well documented in man

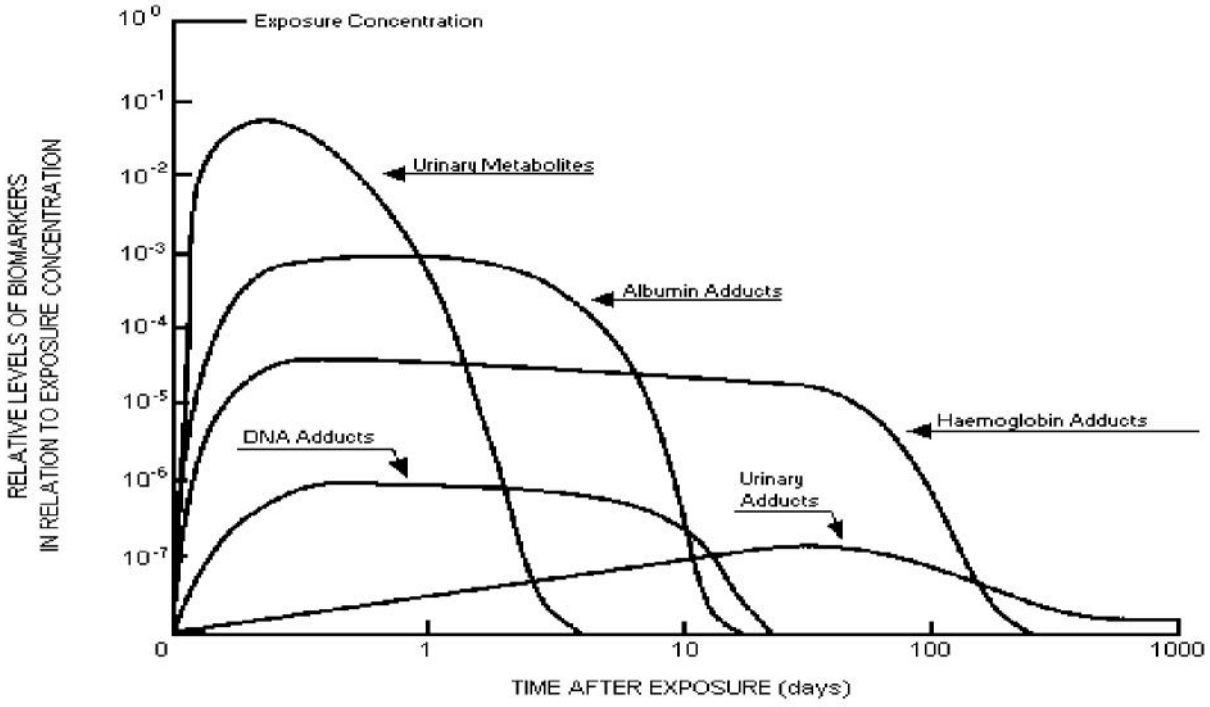

Fig. 3. Hypothetical relationships among different biomarkers of exposure with respect to their relative levels and time of appearance after a single dose (Henderson et al., 1989)

Table 1: Biomarkers used in biological monitaring of human exposure to organochlorines

Compound	Biological indicator	Sample (B, blood, U, urine)
Organochlorines Aldrin and dieldrin	Dieldrin	B
Chlordecone	Chlordecone	B
Chlordane	Chlordane and metabolites	B
Chlorobenzylate	p-p – Dichloroben-zophenone (DBP)	B
DDT	DDT, DDE	B/U
1.3- Dichloro-propene	DCP-MA[a]	U
	Thioethers	U
Endosulfan	Endosulfan	B
Endrin	Endrin Anti-12-hydroxy-en-drin	B U
Heptachlor	Heptachlor epoxide	B
Hexachloroben-zene	Porphyrin pattern	U
Lindane	Lindane	B
Toxaphene	Toxphene	B

[a] DCP-MA. N-acetyl-S-(trans-3-chloroprop-2-enyl)-cysteine and N-cysteine and N-cysteine-S-(cis-3-chloroprop-2-enyl)-cys-tein mercapturic acid.

for dieldrin in blood (approximately 267 days) and DDT in adipose tissue (3.4 years). The biological half-lives of lindane, endrin and chlordane were determined following acute exposures and are estimated to be about 24 h for endrin and 10–20 days for lindane and chlordane. No published data are available on the other OCs, but from animal studies and human data it may be expected that the α-, β-, and δ-isomers of benzene hexachloride and the cyclodiene heptachlor (as heptachlor epoxide) have a long half-life, comparable with dieldrin (Tordoir and Van Sittert, 1994). Exposure to OC pesticides has been investigated in occupationally exposed subjects and in the general population by measuring intact compounds and metabolites in blood, urine, adipose tissue and human milk. Determination of intact OC compounds or their metabolites in blood or serum is a valuable method to monitor short- and long-term exposures to these pesticides, with the exception of endrin. Endrin has a very short half-life in

Table 2: Biomarkers used in biological monitoring of human exposure to organophosphates[a]

Compounds	Biological indicator	Sample (B: Blood; U: Urine)
	ACHE, PCHE NTE Alkylphosphates	B B U
Acephate	Acephate	U
Azinphos-ethyl	Azinphos-ethyl	B
Azimphosmethyl	DMPT	B
Chlorpyrifos	3,5,6-Trichloropyridnol (TCP) DEP, DETP	B/U U
Demeton	DEP, DETP	U
Diazinon	DEP, DETP	U
Dichlorovos	DIP, DMTP	U
Dimethoate	Dimethoate	B
Disulfoton	DETP, DETPh	U
Fenitrothion	3-Methyl-4-nitrophenol (3-4-NP) Fenitrothion	U B
Formothion	Dimethoate	B
Glyphosate	Glyphosate Aminomethyl phosphonic acid	U U
Malathion	DMTP, DMDTP Malathion	U B
Mevinphos	DMP	U
Monocrotophos	DMP	U
Parathion	p-Nitrophenol DEP Parathion/paraxon	U U B
Methylparathion	p-Nitrophenol	U
Phorate	DEP, DETP	U
Quinalphos	DEP, DETP	U
Terbufos	DEP, DETP	U
Trichlorfon	Trichlorfon	B/U
Vaponite	DMP	U

DMTP: dimethylthiophosphates; DEP: diethylphosphate; DETP: diethylthiophosphate; DMP: dimethylphosphate; DETPh: diethyl-phosphorothiolate; DMP: dimethylphosphate; DMDTP: dime-thyldithiophosphate

blood; therefore, determination of its blood concentration is useful only to determine recent high-level exposure. Its major metabolite in urine, anti-12-hydroxyendrin, has been measured in occupationally exposed subjects.

Determination of OC pesticide concentration in adipose tissues was carried out in several studies, mainly on non-occupationally exposed subjects. DDT, dieldrin and heptachor epoxide are the pesticide residues most frequently found in adipose tissues of the general population. Since milk represents an important excretion route for most OC pesticides, the OC concentrations in human milk have been measured in order to estimate the daily intake of infants, mainly in those countries where these pesticides have been used in large amounts both in agriculture and public health. DDT, its main metabolite DDE, hexachlorobenzene, hexachorocyclohexane, dieldrin and heptachor epoxide are the residues most frequently detected in human milk.

Guidelines for biological monitoring

Some studies have investigated the relationship between OC compounds or their metabolites in blood and urine, and the occurrence of toxic effects. Significant dose–effect correlations have been found in several cases. From the results of these studies, biological limit values have been recommended. From the results of these studies, biological limit values have been recommended. In particular, a blood lindane concentration of 20 µg/l and plasma: serum concentrations of 25 µg/l have been indicated as the upper no-effect level for neurological signs and symptoms (DFG, 1998). Absence of induction of liver microsomal enzymes was shown:

- for endrin at urinary anti-12-hydroxyendrin concentrations below 130 µg/g creatinine (Van Sittert and Tordoir, 1987a);
- following repeated exposures to DDT, at DDT and DDE serum concentrations below 250 µg/l (Kolmodin-Hedman, 1974);
- in repeated aldrin and dieldrin exposures, at dieldrin blood concentrations below 100 µg/l (Van Sittert and Tordoir, 1987a).

Organophosphorous (OP) compounds are one of the most important classes of pesticides. They are used as insecticides and, to a lesser extent, as herbicides. Determinations in blood or urine of: (1) unchanged compounds; (2) metabolites deriving from the alkylphosphate moiety of OP molecules; and (3) residues generated by the hydrolysis of the P-X bond have been used to monitor human exposure to some OP compounds. Key information on the OP compounds cited in this article is listed in Table 1. Metabolism of most OP compounds yields alkylphosphates or alkyl-(di)-thiophosphates as terminal products.

Alkylphosphate metabolites are the result of the hydrolysis of the P-X bond in the OP molecule; this reaction is catalysed by enzymes commonly known as A-esterases or phosphoryl-phosphatases, and is present in several mammalian tissues such as liver, plasma and intestine. The alkylphosphates detectable in urine and the main parent compound they can originate from, are listed in Table 2.

Table 3: Alkylphosphates detectable in urine as metabolites of OP pesticides

Metabolite	Main parent compound
Monomethylphosphate (MMP)	Melathion
Dimethylphosphate (DMP)	Dichlorvos, dimethoate, malathion, monocrotophos
Diethylphosphate (DMTP)	Azinphos-methyl, dimethoate
Diethylphosphate (DEP)	Dementen, diazinon
Diethylthiophosphate (DMTP)	Azinphos-methyl, dimethoate
Diethylthiophosphate (DETP)	Diazinon, dementhon
Diethylphosphorothiolate (DEPTh)	Disulfoton, phorate
Dimethyldithiophosphate (DEDTP)	Disulfoton, phorate
Phenylphosphoric acid (PPA)	EPN, Leptophos

Since these metabolites are common to several OP pesticides, this indicator is not specific and is generally used to assess exposure to a group of parent compounds. This

Table 4. Biomakers used in biological monitoring of human exposer to carbamates[a]

Compound	Biological indicator	Sample (B, blood, U, urine)
Carbamates	ACHE, PCHE	B
Aldicarb	Aldicarb-sulfone	U
Carbaryl	1-Napthol	U
	Carbaryl	B
Methomyl	Methomyl	B
Pirimicarb	2DHD, 2MHD	U
Propoxur	2-Isopropoxyphenol (2-IPP)	U
	Propoxur	B

[a]2DHD = 2-dimethylamino-4-hydroxy-5.6-dimethylpyrim-idine; 2MHD=2-methylamino-4-hydroxy-5,6-dimethylpyrim-idine.

practice is helpful when monitoring multiple exposure, but poses a limit to specific toxicological interpretation of the results, since each OP pesticide is characterized by a specific metabolic rate and toxicity level.

Guidelines for biological monitoring

Knowledge about the relationship between exposure and urinary alkylphosphate metabolites is very limited in man. Thus, a reliable estimation of the absorbed dose from urinary alkylphosphate excretion is not possible for most OP pesticides. To date, this has been the major drawback against the use of these bioindicators.

- ❒ Urine DETP concentrations up to 356 µg/g creatinine in post-exposure samples were not associated with neurobehavioral changes (Maizlish *et al.,* 1987).
- ❒ DEP urine concentrations ranging from 0.45 to 1.27 mg/l in morning urine samples were associated with a slight inhibition of PCHE (Weisskopf *et al.,* 1988).
- ❒ No CHE inhibition is observed in subjects with urinary DMTP excretion up to 900 µg/48 h.

Moreover, no adverse effects were reported in a group of manufacturing workers with urinary acephate concentrations up to 5 mg/l (WHO/FAO, 1988). Measurement of 3,5,6-trichloro-pyridinol in urine is a sensitive test to assess chlorpyrifos exposure, at levels insufficient to cause CHE inhibition. For fenitrothion, no CHE inhibition occurs up to urinary 3-methyl-4-nitrophenol concentrations of 3 mg/l, values which are likely to correspond to an oral dose of 7 mg. Urinary determination of glyphosate and its main metabolite, aminomethyl-phosphonic acid, was tentatively used to monitor exposure. Urinary excretion of malathion monocarboxylic acid up to 5 mg/l results in no significant CHE inhibition. For parathion and methyl-parathion, a biological limit value of 0.5 mg/l for p-nitrophenol has been established occupationally exposed workers (ACGIH, 1998).

Similarly, the determination of unchanged compounds or metabolites in blood or urine has been used to monitor human exposure to some carbamate compounds. Table 3. lists the compounds for which data on the use of biological indicators of internal dose in exposed subjects are available in the literature.

For carbaryl, a biological limit value of <10 mg 1-naphthol/l urine for occupational exposure was proposed (WHO, 1975, 1994).

Synthetic pyrethroids (PYR) are a group of insecticides largely used in agriculture and public health because of their relatively low toxicity to man and mammalian species

Table 5: Biomarkers used in biological monitoring of human exposure to pyrethroids

Compound	Biology indicator
Cypermethrin	Cis-and trans 3-(2-2' dichloro-vinyl)-2, 2'-dimethylcyclopropane carboxylic acid (Cl_2CA)
	3-Phenoxybenzoic acid (3-PBA)
Deltamethrin	Deltamethrin
	Dibromovinyl-dimethyl-cyclopropane carboxylic acid (Br_2CA)
Fenvalerate	Fenvalerate
Permethrin	Cis-and trans 3-(2-2' dichloro-vinyl)-2, 2'-dimethylcyclopropane carboxylic acid (Cl_2CA)
B-Cyflutrin	Cis-and trans 3-(2-2' dichloro-vinyl)-2, 2'-dimethylcyclopropane carboxylic acid (Cl_2CA)
A-Cyhalothrin	3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-1-carboxylic acid (CF_3CA)

at the usual application rates and because of their short environmental persistence. The determination in urine of unchanged compounds and metabolites deriving from ester hydrolysis, oxidation and conjugation has been used to monitor human exposure to some PYR compounds. Table 4. lists the compounds for which data on the use of biological indicators of internal dose in exposed subjects have been found in literature.

Dithiocarbamate (DTC) pesticides are mainly used in agriculture as fungicides and, to a lesser extent, as insecticides and herbicides. Additional uses are as biocides for industrial or other commercial applications and in household products. Some DTC are used for vector control in public health. No methods for biological monitoring of

Table 6. Biomakers used in biological monitoring of human exposer to carbamates[a]

Compound	Biological indicator	Sample(B, blood, U, urine)
Carbamates	ACHE, PCHE	B
Aldicarb	Aldicarb-sulfone	U
Carbaryl	1-Napthol	U
	Carbaryl	B
Methomyl	Methomyl	B
Pirimicarb	2DHD, 2MHD	U
Propoxur	2-Isopropoxyphenol (2-IPP)	U
	Propoxur	B

[a]2DHD = 2-dimethylamino-4-hydroxy-5.6-dimethylpyrim-idine; 2MHD=2-methylamino-4-hydroxy-5,6-dimethylpyrim-idine.

exposure to DTC compounds are commonly accepted at present. Determination of DTC metabolites in urine has been carried out in exposed subjects for few compounds. Since DTC are mainly metabolized to carbon disulphide (CS_2), measurement of urine levels of this metabolite has been suggested when monitoring high-level DTC exposure. Some studies investigated ethylenethiourea (ETU) concentrations in urine of workers exposed to EBDTC fungicides. ETU is a metabolite of EBDTC pesticides and it is also the most important substance from a toxicological point of view.

The measurement in urine of the metals present in the DTC molecule has been proposed as an alternative approach to monitor DTC exposure. In particular, urinary excretion of manganese has been investigated in workers exposed to mancozeb. Table 5. lists the compounds for which data on the use of biological indicators of internal dose in exposed subjects have been found in the literature.

Table 7. Biomarkers used in biological monitoring of human exposure to dithiocarbamates and EBDTC (ethylene-bis-dithiocarbamates)

Compound	Biological indicator	Sample (B, blood; U, urine)
Dithiocarbamates	Carbon disulfide (CS_2)	U
	2-Thiothiazolidine-4-carboxylic (2-4-TTCA)	U
Methiram Thiram Ziram	Xanthurenic acid	U
EBDTC (ethylene-bis-dithiocarbamates)	Ethylenethiourea (ETU)	U
	ETU-haemoglobin adducts	B
	Carbon disulfide (CS_2)	U
	2-Thiothiazolidine-4-carboxylic acid (2-4-TTCA)	U
Mancozeb	Mn	U
Maneb	Mn	U

Table 8. Biomarkers used in biological monitoring of human exposure to quaternary ammonuum compounds

Compound	Biological indicator	Sample (B, blood; U, urine)
Diquat	Diquat	B/U
Paraquat	Paraquat	B/U

Amang the quarternary ammonium compounds, diquat and paraquat are widely used as contact herbicides and crop desiccants. They are mainly used as aqueous solutions of their salts. The determination in blood and urine of the unchanged compounds has been used to monitor human

exposure to diquat and paraquat. Table 6. lists the quaternary ammonium compounds (QAC) pesticides for which data on the use of biological indicators of internal dose in exposed subjects have been found in literature.

Diquat concentrations higher than 0.5 in plasma and 1 mg/l in urine samples indicate the absorption of a potentially lethal dose. paraquat urinary concentrations lower than 0.01 mg/l are observed when proper hygiene and safety precautions are adopted.

Coumarin derivatives are used in human drug treatment as anticoagulants. Since they are active in mammalians, they are also used as rodenticides against various species, including rats and mice. Coumarin rodenticides are vitamin K antagonists. Measurement of the activity and/or the concentration of vitamin K-dependent clotting factors (mainly factor II, prothrombin), is the more widely used method for biological monitoring of human exposure to these compounds Measurement of the plasma concentration of Protein Induced by Vitamin K Antagonist:Absence (PIVKA) was recently proposed as a more sensitive biological indicator of coumarin effects on the coagulation system. So far, this test is not commercially available and has only been used for research purposes. In some instances, coumarin exposure was assessed by measuring the plasma concentration of intact compounds (Table 7).

Table 9. Biomarkers used in biological monitoring of human exposure to coumarins

Compound	Biological indicator	Sample(B, blood, U,urine)
Coumarins	Prothrombin	B
	Prothrombin time	B
Brodifacoum	Brodifacoum	B
Bromadiolone	Bromadiolone	B
Chloropha-cinone	Chlorophacinone	B
Difenacoum	Difenacoum	B

Phenoxyacetates (PHE) are widely used as herbicides in agriculture, forestry and, to a smaller extent, in home gardening. The most commonly used are 2,4- dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 4-chloro, 2-methylphenoxyacetic acid (MCPA). 2,4,5-T has been banned in several countries because some commercial formulations were found to be contaminated by 2,3,7,8-tetrachlorodibenzodioxin in the past. At present, the dioxin content of these formulations is reduced to very low concentrations. Table 8. shows most commonly used biomarkers for these pesticides.

Table 10. Biomakers used in biological monitoring of human exposer to phenoxyacetates

Compound	Biological indicator	Sample(B, blood, U,urine)
2, 4-Dichlorophenoxy-acetic acid (2, 4-D)	2, 4-D	B/U
2, 4, 5-Trichlorophe-noxyacetic acid (2,4,5-T)	2,4,5-T	B/U
Dichlorprop 4-Chloro-2	Dichlorprop	U
-methylchlorophe-noxyacetic acid (MCPA)	(MCPA)	B/U
Picloram	Picloram	U
Silvex	Silvex	B/U

Biomarkers of susceptibility

These serve as indicators of a particular sensitivity of individuals to the effect of a xenobiotic or to the effects of a group of such compounds. They can be genetic markers that include alterations in chromosomal structure, such as restriction fragment length polymorphisms (RFLPs), polymorphism of enzyme activities, etc. After the exposure of an organism to a xenobiotic it suffers a biotransformation process in two phases. In the first phase a primary metabolite, usually oxidized and more or less active, originates by the specific action of the microsomal P-450 cytochrome isoenzymic family. In the second phase, the primary metabolite is transformed into another secondary metabolite, which is usually inactive. Some individuals with a low cytochrome P-450 activity will be more resistant to the generation of primary active metabolites, whereas those exhibiting a low activity of enzymes involved in the second phase will show a lower formation of phase II inactive metabolites, increasing the toxicity. Two types of susceptibility biomarkers can be distinguished: polymorphisms activating system markers and polymorphisms of detoxicating systems. Polymorphisms

of activating systems are measurements of the activity of cytochrome P-450 isoenzymes. The family of cytochrome P-450 enzymes is involved in the toxicity of several xenobiotics; associated with the P-450 cytochromes there are a wide range of enzyme activities, referred to as monooxygenase activities. A number of studies have suggested that the various cytochrome P-450 enzymes differ substantially in their amino acid sequences and thus are likely to be encoded by distinct genes. This has been confirmed by comparisons of the complete amino acid sequences of over 71 forms of cytochrome P-450 and of the nucleotide sequences of their corresponding cDNAs and of several genes. A roman numeral corresponding to its specific class designates each form of cytochrome P-450. The most important classes that constitute the different forms of cytochrome P-450 are I–IV. These cytochrome classes comprise several subclasses that are designated by a combination of a letter (A,B,C,D,..) and an identifying arabic numeral, i.e. IA1, IIC8, etc. The most important are IA1 (representing Aryl Hidrocarbon Hydroxylase (AHH) activity), IIC8, IID6 and IIE1. There have been a number of studies trying to establish a relationship between specific cytochrome P-450 activities and some diseases due to environmental toxic exposure, especially cancer. However, there are no definitive conclusions. Markers of polymorphisms of detoxicating systems are measurements of the activity of conjugating enzymes such as glutathione-S-transferases, acetyltransferases, sulfotransferases, glucuronyltransferases and paraoxonase. For instance, predisposition to cancer has been correlated with genetic polymorphisms of N-acetyltransferases. N-Acetyltransferase is an enzyme involved in the deactivation of aromatic amines. After acetylation there is enhanced excretion in urine. In a group of arylamineexposed workers, the slow acetylators are at increased risk of bladder cancer versus rapid acetylators. Another example is glutathione-S-transferase μ, an enzyme involved in the detoxification of reactive metabolites. Half of the population has no functional allele for this enzyme and no or low enzyme activity. These persons are at increased risk of squamous cell carcinoma of the lung. Finally, several organophosphates can be inactivated (hydrolysed) by paraoxonase (PON1). Human paraoxonase exhibits an important polymorphism and in humans three genotypes have been detected: individuals homozygous for the low activity allele; individuals homozygous for the high activity allele; and individuals who are heterozygous. Thus paraoxonase activity can be used as a biomarker of susceptibility to organophosphorus compounds (Mallinckrodt M, 1988) The polymorphism also is observed with the oxons of methyl parathion, chlorthion and ethyl 4-nitrophenyl phenylphosphonate (EPN). However, it is not observed with the oxon of chlorpyrifos. Several pieces of evidence suggest that high levels of serum paraoxonase are protective against poisoning by organophosphorus pesticides whose active metabolites are substrates of this enzyme. Birds, which have very low levels of serum paraoxonase, are very sensitive to parathion, diazinon-oxon and pirimiphos-oxon compared with mammals who have higher levels of this enzyme (Brealey *et al.,* 1980). After injection of partially purified rabbit paraoxonase into rats, an increased resistance to the toxic effects of paraoxon was observed. Recent studies indicate that administration of paraoxonase might have therapeutic value in the case of organophosphate intoxication (Li *et al.,* 1995).

Response or effect biomarkers

Response or effect biomarkers are indicative of biochemical changes within an organism as a result of xenobiotic exposure. The ideal biomarkers should be detected early and be able to show adverse effects before they are irreversible. Those are the most studied biomarkers and they include modifications in some parameters of blood composition, alterations of specific enzyme activities, the appearance of DNA adducts, localized mRNA and protein increases and the appearance of specific antibodies (autoantiboides) against a xenobiotic or a particular cellular fraction (Repetto, 1997) It is noticeable that it is not always easy to distinguish between an exposure and a response biomarker. Perhaps the most typical example is the formation of a DNA adduct—an exposure biomarker whose formation results from the reaction of a xenobiotic with the DNA, which in turn constitutes the cellular response. Moreover, it is evident that a particular response requires a previous exposure to the xenobiotic. Below, we consider some significant examples of response biomarkers.

Nervous system

Despite its obvious importance within toxicology, the area of neurotoxicity seems to be progressing more slowly

Table 11. Suggested relationship between levels of ACHE inhibition and intervention , measures (Van Heemstra-Lequin and Van Sittert, 1986)

Level	Significance	ACHE inhibition	Measures required
First level (No effect)	Values at which no physiological Or biochemical effects are Expected.Values usually found in Normal population without Exposure	_[a]	No action needed
Second Level (Surveillance)	Values indicative of/or compatible with minor and reversible effects	0_30[b], 0_50[c]	Medical surveillance needed. Working conditionsto be exmined to avoid exceeding such a level
Third level (effects)	Values indicative of/or compatible with minor damage (initial symtomps, mild Alterations of sensitive clinical indexes)	30-60[b], 50_70[c]	Temporary removal from exposure and analysis of working conditions needed

[a]By definition, any "effect test" cannot evaluate the first level.
[b]Based on individual pre-exposure baseline.
[c]Based on reference values.

Table 12. Severity and prognosis of acute OP intoxication at different levels of ACHE inhibition

% ACHE inhibition	Level of poisoning	Clinical symptoms	Prognosis
50–60	Mild	Weakness, headache, dizziness, nausea, salivation, lacrimation, miosis, moderate bronchial spasm	Convalescence for 1–3 days
60–90	Moderate	Abrupt weakness, visual disturbances, excess salivation, sweating, vomiting, diarrhoea, bradycardia, hypertonia, tremors of hands and head, disturbed gait, miosis, pain in the chest, cyanosis of the mucous membranes	Convalescence for 1–2 weeks
90–100	Severe	Abrupt tremor, generalized convulsions, psychic disturbances, intensive cyanosis, lung oedema, coma	Death from respiratory or cardiac failure

than other fields with regard to biological monitoring. The complexity of the nervous system and its distinctive peculiarities, together with the problems associated with determination of precise targets for neurotoxic action, are certainly responsible for this limited advancement. Neurochemical measurements for detecting neurotoxicity in humans are limited by the inaccessibility of target tissue. Thus, a necessary approach for identifying and characterizing neurotoxicity is the search for neurochemical parameters in peripheral tissues that are obtained easily and ethically in humans and could represent a marker for the same parameters in nerve tissue (Costa and Manzo, 1995). Perhaps the most significant and useful example of a specific biomarker of neurotoxicity is the inhibition of AChE caused by organophosphorus compounds or carbamate pesticides. The enzyme activity is present in several tissues although inhibition generally is determined from blood samples (whole blood or plasma) and the brain. This biomarker has been used in human toxicology and is studied widely in ecotoxicology (birds, mammals and aquatic species). For example, inhibition of AChE in brain can be taken as proof of mortality in birds, whereas in other animals, such as fish, there is a wider variability of lethal inhibition, in the range of 40–80% (Fairbrother and Bennett, 1988; Minaeu, 1991; Peakall, 1992) For the crustacean group, a large number of publications refer to a concentration-dependent inhibition of ChE with OP and carbamate pesticides (Domingues *et al.,* 2010). This has been documented for *Daphnia magna*, *Gammarus pulex*, *Hyalella azteca*, and *Procambarus clarkii* with several OPs and

carbamates. These observations are in accordance with the expectations based on the mode of action of carbamates and OP pesticides. Attempts have been made to relate ChE inhibition and acute toxicity. Three studies using *D. magna* showed an IC50/LC50 (concentration leading to 50% inhibition/concentration leading to 50% mortality) ratio approximately equal to 1 for the OP malathion, meaning that a concentration resulting in 50% mortality also inhibits ChE by 50%. Nevertheless, this ratio is variable among the different chemicals ranging from 0.3 for acephate to 0.94 for chlorpyrifos in crustaceans. Therefore, it is not possible to establish a relationship between the levels of ChE inhibition and mortality.The decrease in AChE activity in brain may remain for several weeks after the toxic exposure, which is adequately correlated with the effect, in contrast to that occurring in blood with a lower lifespan. Nevertheless, measuring the blood AchE activity has the advantage of easy sampling because there is no need for animal sacrifice.Butyrylcholinesterase (BuChE)—an example of a nonspecific biomarker of neurotoxicity—sometimes is studied in parallel with AChE but its physiological role is unknown and its degree of inhibition is not related simply to toxic effect. Other parameters involved in neurotransmission are the target for a variety of neurotoxicant xenobiotics. These parameters are measured in red blood cells, lymphocytes and fibroblasts (Coasta and Manzo, 1995; Manzo *et al.,* 1996). Several active bioamines are liberated from the nerve ending by exocytosis, a process that is triggered by an influx of Ca2+, and are inactivated by re-uptake and methylation mediated by catechol-O-methyltransferase (COMT). Because of its intracellular localization, monoamine oxidase (MAO) plays a strategic role in inactivating catecholamines that are free within the nerve terminal and not protected by storage vesicles. Isoenzymes of MAO have been characterized with differential substrate specificities; MAO-A preferentially deaminates norepinephrine and serotonin, whereas MAO-B acts on a broad spectrum of phenylethylamines. The MAO-B is a microsomal enzyme and its amino acid sequences from human cerebral cortex and consequently platelets were shown to be identical. Platelet MAO-B activity appears to reflect reliably the enzyme activity in the nervous system. The MAO-B activity is used clinically as a marker the pharmacological effects of MAO inhibitors, such as in the treatment of Parkinson's disease. The MAO-B activity in platelets has been used as a biomarker of the effects of styrene and perchloroethylene occupational exposures,(Checkoway *et al.,* 1994) which are known to cause dopamine depletion. Changes in MAO-B could represent an adaptive response to dopamine depletion and, alternatively, styrene or its metabolite(s) might exert a direct inhibitory effect on the enzyme (Manzo *et al.,* 1996; Mutti and Franchini, 1987). Another example of a neurotoxic biomarker involved in delayed toxicity is the inhibition of neuropathy target esterase (NTE). Several organophosphorus compounds (Mipafox, Methamidofos, etc.), after a single dose, induce delayed neuropathy characterized by symmetrical axonal degeneration, which implicates NTE inhibition and not AChE. Organophosphate-induced delayed neuropathy (OPIDN) is characterized by a lag period of ca. 1–3 weeks, from the moment of intoxication to the appearance of clinical symptoms. The first intoxication was described with TOCP (tri-ortho-cresylphosphate) (Lotti, 1987; Hern´andez-Jerez, 1995) In experimental assays the measurement of NTE in lymphocytes has been used as a biomarker of effect and there is a good correlation between NTE activity in brain and lymphocytes after 24 h of an acute exposure to neurotoxic organophosphorus compounds.

Urinary biomarkers

Long-term exposure to certain nephrotoxic compounds—heavy metals (lead, mercury, cadmium and chromium), halogenated hydrocarbons (chloroform), organic solvents (toluene), therapeutic agents (aminoglucosides, amphotericine B, acetaminophen)—may cause progressive degenerative changes in the kidney.

Table 13.

Serum	
Markers of glomerular	Creating β_2 - microglobulin
Filtration	
Markers of the Glomerular basal	Laminin and anti-GBM antibodies
Membrane (GBM)	
Integrity	
Urine	

Plasma-derived proteins	
High molecular weight	Albumin, transferring
Low molecular weight	β_2- Microglobulin, retinol-binding protein,
	α_1 - microglobulin, clara cell protein, α-amylase
Kidney-derrived	
Components	
Enzymes	Gluathione-S-transferase
	B-N-acetylglucosaminidase
Antigens	
Glomerulus	Fibronectin, laminin
Proximal tubule	Brush border antigens (alkaline phosphatase)
Loop of Henle	Tamm-Horsfall glycoprotein
Others	Glycosaminoglycans, prostanoids

However, because of its large reserve capacity, the clinical signs of renal damage are not apparent until the injury is extensive and consequently irreversible. The prevention of renal disease requires the use of more sensitive tests capable of detecting renal effects at a stage when they are still reversible or at least not so advanced as to trigger a progressive renal disease (Bernard and Lauwerys, 1989) In practice, one usually recommends the determination of at least two plasma-derived proteins in the urine: a high-molecular-weight protein (HMWP) such us albumin for the early detection of a glomerular barrier defect and a low-molecular-weight protein (LMWP) such as retinol-binding protein for the early screening of proximal damage (Nortier *et al.,* 1997) Injury to the kidney can be detected by measuring the urinary activity of kidney-derived enzymes. The lysosomal enzyme β-N-acetyl-D-glucosaminidase (NAG) has been proposed as an index of nephrotoxicity. Advantages of this enzyme include its stability in urine and its high activity in the kidney. The diagnostic value of NAG can be improved further by measuring the B isoenzyme (lesional form released with fragments of cell membranes) (Bernard *et al.,* 1995).

Biomarkers of renal effect

Destruction of renal tissue also can be detected by measuring kidney components in urine that, when they are quantified by immunochemical methods, are referred to as renal antigens. These have been proposed as urinary markers of nephrotoxicity and include: carbonic anhydrase, alanine aminopeptidase and adenosine deaminasebinding protein for the proximal tubule; fibronectin for the glomerulus; and Tamm–Horsfall glycoprotein for the thick ascending limb of the loop of Henle. (Hotz *et al.,* 1995) It is important to realize that this battery of tests does not permit the detection of effects on all areas or segments of the kidney or nephron. No sensitive biomarker is available to detect effects on the deep medulla, the papilla or the distal tubule. Also, there is no biomarker to detect and follow the progression of active fibrotic processes that may insidiously and irreversibly reduce the renal function (i.e. interstitial fibrosis).

Immune system

Direct effects of xenobiotics can affect the immune system and lead to decreased resistance to infections or tumours, alter the course of autoimmunity or induce hypersensitivity reactions.

Full blood count (includes lymphocyte count)

Study of antibody-mediated immunity

- Immunoglobin concentrations in serum (IgM, IgG, IgA, IgE)

Phenotypic analysis of lymphocytes by flow cytometry Surface markers (CD3, CD4, CD8, CD 20, CD 23, etc.)

Study of cellular immunity

- Delayed-type hypersensitivity on skin
- Natural immunity to blood group antigens (anti- A, anti- B)

Autoantibodies and markers of inflammatory response C-Reactive protein

- Autoantibodies to nuclei, DNA and mitochondria

Measure of non-specific immunity

- Interleukins analysis (ELISA or reserve transcription polymerase chain reaction)
- Natural killer cell activity (CD56 or CD60)
- Phagocytosis (chemiluminescence)
- Measurement of complement components

Data of several immunotoxic agents (dioxin, polychlorinated biphenyls, immunotherapeutic drugs, etc.) are derived mainly from animal research (mouse and rat), although a few biomarkers exist that provide specific information on immunotoxicity in humans (Van Loveren *et al.,* 1995) The biomarkers proposed for assessing immunotoxicity in humans are listed in Table 9. and include full blood count, antibody-mediated immunity (immunoglobulin concentrations in serum), phenotypic analysis of lymphocytes by flow cytometry, cellular immunity study, measurement of antibodies and markers of inflammatory response and, finally, examination of non-specific immunity. A variety of factors may modify the immune function, including drugs (non-steroidal anti-inflammatory, vitamin complexes, etc.), biological parameters (gender, age, pregnancy) and other factors (diet, alcohol consumption, circadian rhythms, stress, nutritional state, sleep disturbances, etc.). Within the field of ecotoxicology, the resistance to infection in ducks exposed to organochloride pesticides has been studied by measuring the cellular activities involved in the immune response, particularly the in vitro phagocytic capacity from kidney-isolated macrophages in an number of species.

Biomarkers of immunity

Blood system.

The most studied biomarkers of effect are those related to the alterations of haeme synthesis. The enzyme ALAD is involved in the haeme biosynthetic pathway and the assay is highly specific for lead exposure and effect. The inhibition of ALAD has been shown to be a reliable indicator of the effect of lead in studies on humans and animals (especially several species of fish and birds—eagles, starlings, ducks and geese). One of the most important advantage of this biomarker in ecotoxicology is that animal sacrifice is not required; the effect is slowly reversed, with ALAD values returning to normal only after ca. 4 months (Scheuhammer, 1987; Peakall, 1992; Melancon *et al.,* 1992). Haeme biosynthesis normally is closely regulated and levels of porphyrins are ordinarily very low. Some organochlorines (OCs) cause the formation of excess amounts of hepatic, highly carboxylated porphyrins. The two OCs that are most involved in inducing porphyria in mammals and birds are hexacholorobenzene (HCB) and the PCBs (Walker *et al.,* 1996). Haemoglobin adducts are formed from exposure of several compounds (ethylene oxide, acrylamide, 3-amino-1,4- dimethyl-5OH-pyrido-indole, 4-aminobiphenyl-2,6-dimethylaniline, etc.). Acrylamide is an important neurotoxic agent causing a peripheral neuropathy to experimental animals as well as to humans, and it has been shown to be a potential carcinogen. The conversion rate of acrylamide to glycidamide (reactive metabolite epoxide responsible for neurotoxicity) is significantly correlated with the haemoglobin adducts of acrylamide. These adducts are useful as biomarkers of acrylamide-induced peripheral neuropathy (Bergmark *et al.,* 1991). Because of the relatively long lifespan of the red blood cells (4 months in humans), haemoglobin adducts have been used advantageously for integrating concentrations of genotoxic substances in the blood.

Biomarkers of DNA damage.

At present, many technological approaches permit the detection of covalent interactions of xenobiotics with proteins and other macromolecules. For example, several biomolecules (haemoglobin, serum albumin, etc.) have carboxyl, amino or sulfhydryl reactive groups that can interact with electrophilic compounds. Human DNA adduct formation (covalent modification of DNA with chemical carcinogens) has been shown to correlate with the incidence of a carcinogenic process and is a promising biomarker for elucidating the molecular epidemiology of cancer (Meyer and Bechtold, 1996). There is a sequence of events between the first interaction of a xenobiotic with DNA and consequent mutation: the first stage is the formation of adducts; the next stage may be secondary modifications of DNA, such as strand breakage or an increase in the rate of DNA repair; and the third stage is reached when the structural perturbations in the DNA become fixed and the affected cells often show altered function. One of the most widely used assays to measure chromosomal aberrations is sister chromatid exchange (SCE). Finally, when the cells divide, damage caused by xenobiotics can lead to DNA mutation and consequent alterations in the descent (Shugart , 1996). Some examples of toxics compounds capable of forming human DNA adducts are given in Table 5, including polycyclic aromatic hydrocarbons, aromatic amines, heterocyclic amines, micotoxins and chemotherapeutic agents (Poirier and Weston, 1996). Biological monitoring

to detect human and animal DNA adducts includes 32P post-labelling and recently immunoassays using adduct-

Table 14.

N-Nitrosamines	4-(N-Nitrosomethylamino-1-(3-pyridyl)-1-butanone (NNK) N-Nitrosodimethylamine (NDMA) Diethylnitrosamine (DEN)
Polycyclic aromatic hydrocarbons	Benzo(a)pyrene (BaP) 7,12-Dimethylbenzo(a)anthracene (DMBA)
Aromatic amines	2-Acetylaminoflurane (2-AAF) 4-Aminobiphenyl (4-ABP) 4-Iminobiphenyl (4-IBP)
Heterocylcic amines	2-Amino-3,8-dimethylimidazoquinoxaline
Mycotoxins	Aflatoxin B_1 Ochratoxin A
Chemotherapeutic agents	Cisplatin Mitomycin C Procarbazine Dacarbazine 8-Methoxypsoralen
Others	Ultraviolet light Oxidative damage Malondialdehyde (endogenous)

specific antibodies (Poirier, 1997). They can be detected in blood (lymphocytes), urine or

Biomarkers of DNA damage

tissue homogenates from biopsy (gastric mucosa, liver, etc.), although the study of DNA adducts is not feasible in routine analysis. Damage of DNA has been studied in the field of Ecotoxicology for several marine species (freshwater fish, snapping turtle, etc.) that can be exposed to benzo[a] pyrene (Walker *et al.,* 1996). Future investigations will focus on the implementation and design of studies to assess the association between DNA adduct formation and cancer risk from toxic compounds. Although this association is strongly supported by animal studies, it remains to be ascertained whether adducts also are a necessary component of carcinogenesis in humans. Many studies are being designed now to correlate metabolic polymorphisms, urinary metabolites, chromosomal aberrations and protein and DNA adducts, and it is possible in the future to obtain promising results from the combined use of these biomarkers in the evaluation of cancer risk. Studies available in scientific literature have essentially focused on cytogenetic end-points to evaluate the potential genotoxicity of pesticides in occupationally exposed populations, including pesticide manufacturing workers, pesticide applicators, floriculturists and farm workers (Bolognesi, 2003). A positive association between occupational exposure to complex pesticide mixtures and the presence of chromosomal aberrations (CA), sister-chromatid exchanges (SCE) and micronuclei (MN) has been detected in the majority of the studies, although a number of these failed to detect cytogenetic damage. Conflicting results from cytogenetic studies reflect the heterogeneity of the groups studied with regard to chemicals used and exposure conditions. Genetic damage associated with pesticides occurs in human populations subject to high exposure levels due to intensive use, misuse or failure of control measures. The majority of studies on cytogenetic biomarkers in pesticide-exposed workers have indicated some dose-dependent effects, with increasing duration or intensity of exposure. Significant simultaneous increase in CA and SCE was observed in workers involved in the production of mancozeb formulation containing fungicide and in CA in herbicide production workers exposed to 2,4, 5-T and 2,4-dichlorophenoxy acetic acid (2,4-D). Organophosphate exposure was also associated with an increase in cytogenetic damage as SCE frequency positive results were reported in workers employed in insecticide production plants.

Biomarkers as a tool for monitoring pollution

Researchers have tried to apply suitable biomarker containg appropriate bioindicator to monitor pesticide pollution. The common pasture earthworm (*Aporrectodea caliginosa*) contains cholinesterase (ChE) and glutathion S-transferase (GST) (Booth *et al.,* 2001). But cholinesterase (ChE) is found to be much more sensitive to unused pesticide, chlorpyriphos than glutathion S-transferase (GST). Thus *Aporrectodea caliginosa* can be used as a

Table 15.

Study subjects (exposed/ controls)	Exposure	Duration (years)	Analyzed biomarkera	Result	References
44/30	Novozir Mn 80 (mancozeb-contained fungicide)	Upto 2	CA SCE	Pos(+1.83) Pos(+1.17)	Jablonika *et al.*
14/50, nine formulators, five packers 19/36	Azynphos methyl, dimethoate, malathion, methyl parathion	N. D. 10-30	SCE CA	Pos(+1.21) Pos(+2.05)	Laurent *et al.* Kaioumova and Khabutdinova
20/20	Pesticide mixture; most commonly used pesticides; 2,4-D, atrazine, alachlor, cyanazine, malathion	4-30 (sampling carried out after 8 months high exposure period)	CA MN	Pos(+6.10) Pos(+3.63)	Graj-Vrhovac and Zeljezic, Zeljezic and co-workers
20/20			SCE	Pos(+2.23)	Zeljezic and Graj-Vrhovac
135/111	Organophosphates	1-24	SCE	Pos(+1.85 smokers) Pos(+1.63 non-smokers)	Padmavati et al.

bioindicator of pesticide pollution. The phytoplankton is a model of choice for the study of the long term effects of pollutant exposure at population level (Akcha *et al.,* 2008). As primary producers, phytoplankton constitutes the first level of marine trophic chains. Due to its microscopic size, it is possible to get sample at population and community levels. Some species can be cultivated in photobioreactors under controlled conditions. Due to a high growth rate, phytoplankton offers the possibility to study the trans-generational effects of pollutant exposure. The phytoplankton strain *Karenia mikimotoi* (GATIN95) isolated in 1995 in the bay of Brest, France, can be used as a bioindicator for pesticide pollution in aquatic environment. Endosulfan exposure resulted in DNA damage for *K. mikimotoi* nuclei. Genotoxicity was observed from 1 µg/L of endosulfan and was not concentration dependent. Brain acetylcholinesterase (AChE) of some Wshes from the coast of Rio de Janeiro State was studied as a possible pesticide biomarker in marine environmental monitoring (Oliveira *et al.,* 2007). AChE specific activity in brain varied from 145 to 530U/g of proteins and the Michaelis–Menten constant (KM) for acetylthiocholine varied from 104 to 291µM among the 20 species studied. The enzyme sensitivity to methyl paraoxon, evaluated by the inhibition kinetic constants, shows that some species (*Paralonchurus brasiliensis* and *Genidens genidens*) are more sensitive (IC50-30min= 455 and 468nM, respectively). It has been found that, the brain AChE levels and its sensitivity to organophosphorus in fishes of the Rio de Janeiro coast are quite variable. Further *in vitro* and *in vivo* studies on the properties of the brain AChE present in each fish species will make it easier to choose marine sentinel species as a biomarker. However, this study have shown that a sentinel Neotropical fish species should have the highest brain AChE level among the more sensitive ones.

The case studies above provide preliminary examples of when and how biomarkers can be used to infer the source and magnitude of exposure among a set of competing sources and pathways. The answer to this question is chemical specific and relates to how well the biomarker matches the characteristics of an “ideal” biomarker, in particular, ease of collection and persistence. For example, the dioxins and polychlorinated biphenyls, which are persistent in biological organisms, facilitate biomonitoring that has enabled scientists and public health professionals to track population trends and to evaluate progress in

reducing exposures. To some extent, biomarkers of dioxin-like substances have also been useful in demonstrating for these compounds the relative importance of global versus regional and local source as well as important contributions through food rather than inhalation pathways. In contrast, biomarkers of a compound that is metabolized relatively quickly provide only limited opportunity for inferring sources or exposure pathways. Biomarkers for OPs are somewhere between the extremes of dioxinlike compounds and rapidly metabolized compounds in providing an opportunity to explore source and pathway contributions. OPs can be measured directly in blood (and possibly in urine) and produce both generic metabolites—DAPs—and OP-specific (or near-specific) biomarkers such as TCPy. The use of these biomarkers in combination provides a better opportunity to disaggregate both source and pathway contributions than is possible for a rapidly metabolized compound. However, little has been done to explore the capabilities and limitations of using multiple biomarkers in combination to infer exposure attributes. One example discussed is the direct intake of the biomarker itself giving rise to an over-estimate of exposure; there are likely others.

Important goals for near-term biomarker research must include systematic efforts across a broad range of chemical substances to determine the reliability of biomarkers to infer the source and exposure pathway in cumulative risk assessments. This may be done most effectively through the simultaneous collection of biomarker data of various types on the same individuals, populations, or ecosystems. In conclusion, the public health goal of quantifying the burden of disease that is attributable to the cumulative impacts of environmental exposure remains elusive. However, the steady progress in development of biomarkers of exposure, susceptibility, and effect, coupled with emerging technologies for environmental monitoring, offers unprecedented opportunities to examine and prevent cumulative health risks and to redefine approaches to environmental protection.

Conclusion

Biomarkers are used to detect the effects of pesticides before adverse clinical health effects occur. Pesticides and their metabolites are measured in biological samples, serum, fat, urine, blood, or breast milk by the usual analytical techniques. Biochemical responses to environmental chemicals provide a measure of toxic effect. A widely used biochemical biomarker, cholinesterase depression, measures exposure to organophosphorus insecticides. Techniques that measure DNA damage (e.g., detection of DNA adducts) provide a powerful tool in measuring environmental effects. Adducts to hemoglobin have been detected with several pesticides. Determination of chromosomal aberration rates in cultured lymphocytes is an established method of monitoring populations occupationally or environmentally exposed to known or suspected mutagenic-carcinogenic agents. Biomarkers will have a major impact on the study of environmental risk factors. The basic aim of scientists exploring these issues is to determine the nature and consequences of genetic change or variation, with the ultimate purpose of predicting or preventing disease.

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References

Akcha, F., Arzul, G., Rousseau, S. and Bardouil, M. 2008. Comet assay in phytoplankton as biomarker of genotoxic effects of environmental pollution. *Mar. Environ. Res.* **66**: 59-61.

American Conference of Governmental Industrial Hygienists (ACGIH) 1998. TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents. ISBN 1-88-2417-23-2. ACGIH. Cincinnati, OH.

Bergmark, E., Calleman, C.J. and Costa, L.G. 1991. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol. Appl. Pharmacol.* **111**: 352–363.

Bernard, A. and Lauwerys, R. 1989. Epidemiological application of early markers of nephrotoxicity. *Toxicol. Lett.*; **46**: 293–306.

Bernard, A., Thielemans, N., Roels, H. and Lauwerys, R. 1995. Association between NAG-B and cadmium in urine with no evidence of a threshold. *Occup. Environ. Med.* **52**: 177–180.

Bolognesi, C. 2003. Genotoxicity of pesticides. *Mutat. Res.* **543**: 251-272.

Booth, L.H., Hodge, S. and O'Halloran K. 2001. Use of biomarkers in earthworms to detect use and abuse of field applications of a model organophosphate pesticide. *Bull. Environ. Contam. Toxicol.* **67**: 633-640.

Brealey, C.J., Walker, C.H and Baldwin, B.C. 1980. A-esterase activities in relation to differential toxicity of pirimiphos-methyl to birds and mammals. *Pest. Sci.* **11**: 546–554.

Checkoway, H., Echeverria, D., Moon, J.D., Heyer, N. and Costa, L.G. 1994. Platelet monoamine oxidase B activity in workers exposed to styrene. *Int. Arch. Occup. Environ. Health* **66**: 359–362.

Costa, L.G. and Manzo, L. 1995. Biochemical markers of neurotoxicity: research strategies and epidemiological applications. *Toxicol. Lett.* **77**: 137–144.

Deutsche Forschung-Gemeinschaft (DFG) 1998. List of MAK and BAT Values 1998. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report No. 34. VCH, Weinheim.

Domingues, I., Agra, A.R., Monaghan, K., Soares A.M.V.M., and Nogueira A. J.A. 2010. Cholinesterase and glutathione-s-transferase activities in freshwater invertebrates as biomarkers to assess pesticide contamination. *Environ. Toxicol. Chem.* **29**: 5-18.

ENTOX/TIWET (The Faculty of the Department of Environmental Toxicology and The Institute of Wildlife and Environmental Toxicology, Clemson University). 1996. Aquatic and Terrestrial Ecotoxicology. In Casarett and Doull's Toxicology. The Basic Science of Poisons Klaassen CD (ed.). McGraw-Hill: New York,; 883–905.

Fairbrother, A. and Bennett, J.K. 1988. The usefulness of cholinesterase measurement. *J. Wildl. Dis.* **24**: 587–590.

Geldmacher-von Mallinckrodt, M. and Diepgen, T.L. 1988. The human serum paraoxonase—polymorphism and specificity. *Toxicol. Environ. Chem.* **18**: 79–196.

Graham, D., Henderson, F.W. and House, D. 1988. Neutrophil influx measured in nasal lavages of humans exposed to ozone. *Arch Environ Health,* **43**: 228-233.

Hern´ andez-J ´ erez, A.F. 1989. Neurotoxicidad retardada inducida por organofosfatos (OPIDN): alteraciones de la glucolisism durante la fase de desarrollo. Estudio in vitro e in vivo. Doctoral Thesis, Universidad de Granada. Granada.

Hotz, P., Lorenzo, J., Fuentes, E., Cortes, G., Lauwerys, R. and Bernard, A. 1995. Subclinical signs of kidney dysfunction following short exposure to silica in the absence of silicosis. *Nephron* **70**: 438–442.

Kolmodin-Hedman, B. 1974. Exposure to lindane and DDT and its effects on drug metabolism and serum lipoproteins. Stockholm, Departments of Clinical Pharmacology (at Huddinge Hospital) an Pharmacology, Karolinska Institute, Toxicology (Swedish Medical Research Council) and Division of Occupational Medicine, National Board of Occupational Safety and Health (Thesis).

Li, W.F., Furlong, C.E. and Costa, L.G. 1995. Paraoxonase protects against chlorpyrifos toxicity in mice. *Toxicol. Lett.* **76**: 219–226.

Lotti, M. 1987. Organophosphate-induced delayed polyneuropathy in humans: perspectives for biomonitoring. *Trends Pharmacol. Sci.* **8**: 175–176.

Maizlish, N., Schenker, M., Weisskopf, C., Seiber, J. and Samuels, S. 1987. A behavioral evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. *Am. J. Ind. Med.* **12**: 153–172.

Manzo, L., Artigas, F., Mart´ınez, E., Mutti, A., Bergamaschi, E., Nicotera, P., Tonini, M., Candura, S.M., Ray, D.E. and Costa, L.G. 1996. Biochemical markers of neurotoxicity. A review of mechanistic studies and applications. *Hum. Exp. Toxicol.* **15**: S20–S35.

Melancon, M.J., Alscher, R., Benson, W., Kruzynski, G., Lee, R.F., Sikka, H.C. and Spies, R.B. 1992. Metabolic products as biomarkers. In Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress, Hugget, R.J., Kimerle, R.A., Mehrle Jr, P.M., Bergman, H.L. (eds). Lewis: Chelsea, 87.

Meyer, M.J. and Bechtold, W.E. 1996. Protein adduct biomarkers: state of the art. *Environ. Health Perspect.* **104**: 879–882.

Mineau, P. 1991. Cholinesterase Inhibiting Insecticides: their Impact on Wildlife and the Environment. Elsevier Science: Amsterdam.

Mutti, A. and Franchini, I. 1987. Toxicity of metabolites to dopaminergic systems and the behavioural and neuroendocrine effects of organic solvents. *Br. J. Ind. Med.* **44**: 721–723.

Nortier, J., Deschodt-Lanckman, M., Simon, S., Thielemans, N., De Prez, E., Depierreux, M., Richard, C., Lauwerys, R., Bernard, A. and Vanherweghem, J.L. 1997. Proximal tubular injury in Chinese herbs nephropathy: monitoring by neutral endopeptidase and microprotein measurements. *Kidney Int.* **51**: 288–293.

Oliveira, M.M., Silva Filho, M.V., Cunha Bastos, V.L.F., Fernandes, F.C. and Bastos, J.C. 2007. Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes, *Marine Envioron. Res.* **63**: 303–312.

Peakall, D. 1992. Biomarkers of the nervous system. In Animal Biomarkers as Pollution Indicators, Peakall, D. (ed.). Chapman and Hall: London, 19.

Peakall, D. 1992. Thyroid function, retinols, haem and regulatory enzymes. In Animal Biomarkers as Pollution Indicators, Peakall, D. (ed.). Chapman and Hall: London, 108.

Poirier, M.C. and Weston, A. 1996. Human DNA adductmeasurements: state of the art. *Environ. Health Perspect.* **104**: 883–893.

Poirier, M.C. 1997. DNA adducts as exposure biomarkers and indicators of cancer risk. *Environ. Health Perspect.* **105**: 907–912.

Repetto, M. 1997. Diagn ´ ostico de la intoxicaci ´ on. In Toxicolog´ıa Fundamental, Repetto, M. (ed.). *D´ıaz de Santos*: Madrid, 327–333.

Scheuhammer, A.M. 1987. Erythrocyte delta-aminolevulinic acid dehydratase in birds. I. The effects of lead and other metals in vitro. *Toxicol.* **45**: 155.

Silbergeld, E.K. and Davis, D.L. 1994. Role of biomarkers in identifying and understanding environmentally induced disease. *Clin. Chem.*; **40**: 1363–1367.

Tordoir, W., Van Sittert, N.J., 1994. Organochlorines. In: Tordoir, W., Maroni, M., He, F. (Eds.), Health Surveillance of Pesticide Workers. A Manual for *Occupational Health Profes. Toxicol.* **91**: 51-57.

Van Loveren, H., Steerenberg, P.A. and Vos, J.G. 1995. Early detection of inmunotoxicity: from animal studies to human biomonitoring. *Toxicol. Lett.* **77**: 73–80.

Van Sittert, N.J. and Tordoir, W.F. 1987a. Aldrin and dieldrin. In: Alessio, L., Berlin, A., Boni, M., Roi, R. (Eds.), Biological Indicators for the Assessment of Human Exposure to Industrial Chemicals. Commission of the European Communities, Luxemburg, pp. 7–21.

Walker, C.H., Hopkin, S.P., Sibly, R.M. and Peakall, D.B. 1996. Biomarkers. In Principles of Ecotoxicology, Walker, C.H., Hopkin, S.P., Sibly, R.M., Peakall, D.B. (eds). Taylor and Francis: London, 175–194.

Weisskopf, C.P., Seiber, J.N., Maizlish, N. and Schenker, M. 1988. Personnel exposure to diazinon in a supervised pest eradication program. *Arch. Environ. Contam. Toxicol.* **17**: 201–212.

WHO 1975. Data sheet on pesticides, No. 1: Endrin. WHO, Geneva.

WHO 1994a. Data sheet on pesticides, No. 72: Azinphos-Ethyl. World Health Organization: Food Agriculture Organization, Geneva.

WHO/FAO 1988. Pesticide residues in food. Report of the 1987 Joint Meeting of the FAO panel of experts on pesticide residues in food and the environment and the WHO expert group on pesticide residues. WHO, Geneva.