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Pesticide Biomarkers

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1. Introduction

Biomarkers were originally identified in the field of human medicine and were first promoted for use in ecotoxicology in the early 1990s. The simplest and most often-used definition of a biomarker is the one devised by David Peakall: "a biological response to chemicals that give a measure of exposure and sometimes, also of toxic effect". However, the term biomarker and the related term bioindicator have been defined and redefined by many different researchers and institutions (Peakall, 1994).

The National Academy of Science, in 1987, defined a biomarker as "a xenobiotically induced variation in cellular or biochemical components or processes, structures, or function that is measurable in a biological system" (Huggett et al., 1992). The term is most often employed to refer to molecular, physiological, and organismal responses to contaminant exposure that can be quantified in organisms inhabiting or captured from natural systems. A response that is limited to laboratory studies falls outside the generally held concept of a biomarker.

The use of biomarkers has great potential to complement the current methods used to determine the presence and potential impact of environmental pollutants. However, chemical analysis is expensive and is applicable only to a small percentage of environmental contaminants. Acceptable and critical contaminant levels have been established for only a few compounds, so determination of only the concentration does not really provide any information on the ecological hazards. In addition, chemical concentrations do not account for the complexity of the systems involved and provide little meaningful information on the possible effects of the contamination on the organisms. It is difficult to predict such effects based on chemical concentration alone due to variable environmental factors such as pH, temperature, and moisture. These factors impact the contaminant form, its movement through the environment and its ultimate uptake by organisms, thereby impacting the ultimate toxicity. To address these issues, chemical contaminant concentration analysis has been supplemented with the use of both acute and chronic biomonitoring in which live organisms are subjected, in a laboratory setting, to varying amounts of contaminants and observed for toxic effects. The primary disadvantage of these tests lies with the established trend to use only a few types of organisms that are easily handled and maintained in the laboratory. This limits the validity of the application of information obtained from disparate species.

Many scientists recommend that biomarkers be used as an additional ecotoxicology assessment method based on the concept that contaminant-based changes at molecular,

cellular, and genetic levels occur in response to the stress caused by the action of human-introduced contaminants, thereby giving advanced warning of future ecosystem damage. Biomarker study results cannot always confirm the presence of an exact chemical but instead give an indication of the presence of contamination by a class of chemicals. This finding provides evidence that further, and more expensive, chemical analyses are warranted (Peakall, 1994; van Gestel and Brummelen, 1996).

Biomarkers or biological markers are molecular-, biochemical-, or cellular-level indicators in either wild populations taken from contaminated habitats or in organisms experimentally exposed to pollutants that indicate that such organisms have been exposed to toxic chemicals and the magnitude of the organism's response to the contaminant. Biological markers measured in wild animals can directly contribute to the detection, quantification, and understanding of the significance of the exposure to chemicals in the environment. These measurements in environmental species may also help to assess the potential for human exposure to environmental pollutants and to predict human health risks (Shugart, 2005).

Another practical use for biomarkers is the detection and quantification of prior or ongoing exposures to specific chemicals; biomarkers have been successfully used in biological monitoring programs in industry but have only recently been used to monitor environmental exposures. Medical researchers are seeking biomarkers that can be employed to i) detect early stages of a disease to enhance successful intervention; ii) determine the effectiveness of intervention strategies; and iii) detect cells at risk from a toxicant. Finally, research is ongoing, particularly in the field of genetics, to find inherited biomarkers of susceptibility that can be used for the detection and protection of sensitive populations (Klaassen, 2008).

The specificity of biomarkers to chemicals varies greatly. Both specific and nonspecific biomarkers have their place in environmental assessment. A nonspecific biomarker can demonstrate that a pollutant is present in a meaningful concentration but does not indicate which particular chemical is present. Based on such information, a more detailed chemical investigation could be justified. In contrast, specific biomarkers indicate that a specific chemical is present but give no information on the presence of other chemicals (Shugart, 2005).

A list of criteria for the evaluation of biomarkers should include the following: a) Biological specificity. It is important to know to which species or classes a biomarker is relevant. The inhibition of the enzyme acetylcholinesterase (AChE) by organophosphate (OP) and carbamate (CAR) pesticides can be applied throughout the animal kingdom, whereas the induction of vitellogenin is confined to those vertebrates that lay eggs; b) Clarity of interpretation. One should be able to clearly distinguish whether a stress is natural or anthropogenic. It is valuable to know the mechanism of response to the chemical in assessing this point; c) Time of response. The temporal expression of different biomarkers can vary widely from instantaneous to years. Depending on the type of study, a slow or a rapid manifestation may be desirable; d) Permanence of response. It is important to know how long the response lasts. If it is transient, it may be easily missed. The inhibition of AChE, especially in blood, is a transient response, and thus it is necessary to know when the exposure occurred to assess the importance of the degree of inhibition. In contrast, the inhibition of the enzyme amino levulinic acid dehydratase by lead is only slowly reversed; e) Reliability. This criterion can be considered in two ways: 1) environmental influences that modulate an organism's response to a chemical, and 2) inherent variations in the biological response to a given exposure. To have a reliable biomarker, it is important to know the extent of all variations; f) Methodological considerations. Important considerations include the precision (analytical reproducibility of the method), cost, and ease of analysis. Although

many reliable assays have been developed, there is a need for standardization, similar to that used in analytical chemistry, so that the results from different laboratories can be comparable; g) Relative sensitivity. It is important that the biomarker be sensitive when compared to other endpoints, such as mortality or reproductive impairment, and it is important to know the relative sensitivity of this comparison; h) Validation in the field. For a biomarker to be useful in environmental assessment, it must be validated in the field. Organisms in the field are subjected to a wide range of variables that are usually accounted for or controlled in laboratory experimentation; i) Linkage to higher-level effects. A biomarker is more useful if there is clear linkage to an effect at higher levels of organization. Studies on invertebrates have been particularly fruitful, as population changes among such species occur more rapidly than in higher species (Shugart, 2005).

Biomarkers have an advantage over chemical analysis in that they can demonstrate whether or not an organism has been meaningfully exposed. For some classes of persistent organic chemicals, such as the organochlorines, current detection limits are as low as parts per trillion. Thus, these man-made chemicals can be detected in almost all samples, but the physiological significance is rarely known. With biomarkers, it is possible to determine whether the physiology of the organism is significantly different from normal. If it is, then the organism is considered to be meaningfully exposed. Equally important, if the physiology is not significantly different, then the organism is considered not to be meaningfully exposed, even if the chemical(s) can be detected. The ability to determine whether or not an organism is meaningfully exposed is important in deciding whether regulatory action should be taken or in determining whether or not remedial action has been successful.

Biomarkers are generally divided into three categories as markers of exposure, effect, and susceptibility. Each of these types of biomarkers is described below:

Biomarkers of exposure are measures of internal substances and thus reflect various manifestations of the internal doses that result from exposure. Markers of interest include those that provide measures of the i) total internal dose (such as the blood, urine, or breath level of a chemical); ii) dose to a target organ (which may be in the form of a macromolecular adduct formed between the chemical or its metabolite and the organ tissue); or iii) biologically effective dose (which can only be measured if the mechanism of disease induction is known in sufficient detail to suggest what entities might represent the biological effect).

Biomarkers of effect are any changes in a biological system that reflects qualitative or quantitative impairment resulting from exposure. While a distinction is made between biomarkers of exposure and biomarkers of effect, in practice the two areas overlap. For example, DNA adducts may be biomarkers of exposure, but if they occur at specific sites known to induce mutations leading to cancer, the adducts may also be biomarkers of effect.

Biomarkers of susceptibility. Indicators of individual or population differences that influence the response to environmental agents are called "biomarkers of susceptibility." These indicators might include such characteristics as an enhanced metabolic capacity for converting a chemical to its reactive, more toxic, metabolites or differences in the number of receptor sites that are critical for a specific response. An example is the inherited deficiency in the enzyme α-1-antitypsin, which is associated with an increased susceptibility to the development of emphysema. New assays developed by researchers in the field of toxicogenomics allow for the detection of genetic polymorphisms that can affect the susceptibility to pollutant exposure. Such markers can be quite valuable in providing

information that can contribute to the protection of susceptible populations. Knowledge of the mechanisms of susceptibility can be important in designing the therapy for a disease. However, the use of such markers is fraught with legal and ethical problems, as the identification of persons with enhanced susceptibility to adverse health effects from exposure to chemicals could lead to discrimination against those persons in obtaining jobs and insurance (Henderson, 2005).

1.1 Biomarkers and pesticides

Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality. Pesticides are substances used to control pests such as insects, water weeds, and plant diseases. Naturally occurring pesticides have been used for centuries, but the widespread production and use of modern synthetic pesticides did not begin until the 1940s. When pesticides enter aquatic systems, the environmental costs can be high. Unintentional pesticide-related fish kills occur. Some of these kills have been large, involving thousands of fishes as well as frogs, turtles, mussels, water birds, and other wildlife. Fish and other wildlife species, including rare and endangered ones, such as the peregrine falcon, bald eagle, and osprey, have been victims of pesticide poisoning. Pesticide use is one of many factors contributing to the decline of fish and other aquatic species (Helfrich et al., 2009). The initial efforts to monitor pesticide exposure in organisms focused on the major plasma esterases in humans, as such targets can be inhibited and modified by some pesticides (Black et al., 1999; Peeples et al., 2005), although investigators now indicate than the identification and characterization of other biomarkers is necessary (Kim et al., 2010).

2. Biomarkers of pesticide exposure in aquatic organisms and human populations

2.1 Esterase inhibition as a biomarker of pesticide exposure in aquatic organisms

Recently, several studies have evaluated AChE and butyrylcholinesterase (BChE) activities as biomarkers of the exposure to OP in different aquatic organisms from contaminated areas in several countries. Tlili et al. (2010) found an inhibition of AChE activity in the bivalve *Donax trunculus* from a polluted site (Radès Méliane) compared to that from a reference site (Sidi Jehmi) in the Gulf of Tunis (Tunisia). Bernal-Hernández et al. (2010) showed than AChE activity was 65% lower in another bivalve, *Crassostrea corteziensis*, from Boca de Camichín than in control oysters, in a subtropical Mexican Pacific estuary, suggesting the presence of OP and CAR pesticides in these aquatic environments. In a similar study performed in Argentina, Attademo et al. (2011) reported that BChE activity was lower in a native frog, *Leptodactylus chaquensis*, from rice fields where pesticides such as methamidophos (OP), cypermethrin (pyrethroid) and endosulfan (organochloride) were used, as compared to those from a reference site. In contrast, Printes et al. (2011) did not observe an association of cholinesterase (ChE) activity in *Chironomus xanthus* with exposure to sediments containing pesticides from Monjolinho River (Southeast Brazil); the authors suggested that the selected biomarker was not sensitive and specific enough to detect the effects of pesticide contamination at the levels measured in the study area. As in aquatic organisms, several studies around the world have evaluated the exposure to OP by measuring cholinesterase activity.

2.2 Esterase inhibition as a biomarker of pesticide exposure in human populations

The inhibition of AChE activity has been observed in subsistence farmers from rural communities of Campeche, Mexico (Rendón von Osten et al., 2004), and in young children working on Mexican tobacco plantations in Nayarit, Mexico (Gamlin et al., 2007); BChE activity was also shown to be inhibited in Tunisian agricultural workers (Araoud et al., 2010).

However, OP exposure has been assessed using a blood cholinesterase test in which a reduction on AChE or BChE activity indicates exposure to OP (Ellman et al., 1961). BChE assays are used for the early and acute effects of exposure to OP because AChE is less sensitive (Wilson et al., 1996). However, BChE has limited utility due to its 11-day half-life in plasma (Richards et al., 2000), as opposed to AChE membrane-bound protein on red blood cells, which has a lifespan of 120 days. AChE inhibition has been used as one standard method to detect OP exposure (Holmstedt, 1959), but this assessment has disadvantages, as intra- and inter-subject variabilities are about 10% in the same person and about 10-40% among subjects (Lotti, 1995), respectively. The integrated use of several biomarkers, such as cholinesterases and others, may be necessary for biomonitoring programs to diagnose pesticide exposure in wild populations (Attademo et al., 2011). Thus, it was proposed that other biomarkers should be identified and characterized, such as the esterase identified as acetyl peptide hydrolase (APH), which was inhibited by some OP (Quistad et al., 2005). Similarly, Noort et al. (2009) suggested that the affinity of OP for albumin could provide a mechanism for a more complete assessment of OP pesticide exposure. In addition, a mass spectrometry method to identify exposure to several pesticides (dichlorvos, chlorpyrifos oxon and aldicarb) based on the identification of pesticide adducts on the active site (i.e., serine) of human BChE has been proposed by Li et al. (2010a).

2.3 Analytical determinations

Biomonitoring helps to identify new exposures to chemicals, trends in exposure, the distribution of chemicals in the population and particularly vulnerable groups, and it is a tool for scientists as well as for policy makers (Angerer et al., 2007). Many authors have monitored the levels of pesticides as persistent organic pollutants (POPs) in ecological studies. Zapata-Pérez et al. (2007) reported the presence of HCHs, DDTs and chlordanes in ariidae *Ariopsis felis* (Linnnaeus, 1766) in three ecosystems in the Southern Gulf of Mexico, and contaminants were higher in Laguna de Terminos than in Celestun and Dzilam. In a different study, p,p´-DDE, toxaphene, total chlordanes, dieldrin, dacthal, endosulfan, gamma-HCH and methoxychlor were detected in fish from the Colorado River and its tributaries (Hinck et al., 2007). The direct measurement of pesticides is a challenge; for example, OP quantification is a challenge due to its rapid metabolism in organisms and its breakdown in the environment, thus the estimations are rough or involve the identification of OP metabolites or degradation products (Barr et al., 2005).

Garabrant et al. (2009) reported a negative relation between urinary 3,5,6-tricholo-2-pyridinol (TCPy) and BChE in workers occupationally exposed to chlorpyrifos. As approximately 75% of OP yield dialkylphosphates (DAPs), gas chromatography coupled with mass spectrometry (GC-MS) has been employed to detect these primary metabolites in urine (Barr et al., 2005). Cocker et al. (2002) reported than the DAPs found in urine from workers potentially exposed to OP were lower and were unlikely to cause a significant reduction of AChE. One study of immigrant Latino farm workers reported that DAPs were not associated with hazardous work conditions (Grzywacz et al., 2010). Another study in

Latino farmers (mostly from Mexico) in eastern North Carolina, in which determinations of DAPs were conducted using urine, blood and saliva samples taken several times monthly, showed variability in the DAPs frequencies, and the authors indicate the importance of longitudinal studies in such populations (Arcury et al., 2009). Other studies with agricultural workers from Mexico have evaluated the presence of DAPs in urine (Lacasaña et al., 2010; Recio et al., 2001, 2005).

Recently, Tsatsakis et al. (2010) proposed a new, simple and fast method to evaluate DAPs in human head hair by GC-MS, in which the metabolites were detected in individuals with occupational exposure. A sensor that provided a rapid, clinically accurate and quantitative tool for TCP detection showed great promise for testing a metabolite biomarker (3,5,6-trichloropyridinol) in humans exposed to pesticides (Zou et al., 2010).

Sunyer et al. (2010) reported the presence of p,p´-DDE, hexachlorobenzene and beta-hexachlorocyclohexane in the serum of pregnant women in the first trimester from a general population in Catalonia, Spain. Similarly, the presence of p,p´-DDE was observed in pregnant women in Greenland (Inuit, Kharkiv and Warsaw mothers) by Wojtyniak et al. (2010). In a similar study, Chevrier et al. (2011) observed quantifiable levels of azatrine or atrazine mercapturate and dealkyllated and hydroxylated traizine metabolites in the urine of pregnant women from the Britany region. Pesticide exposure has been associated with illnesses such as diabetes and pre-diabetes; heptachlor epoxide, oxychlordane, intermediates for p,p´-DDT, beta-hexachlorocyclohexane, p,p´-DDE and trans-nonachlor were related to diabetes in the National Health and Nutrition Examination Survey (NHANES) (Everrett and Matheson, 2010). In patients with exocrine pancreatic cancer, the years worked in agriculture did not associate with the p,p´-DDT, p,p´-DDE, hexachlorobenzene or β–hexachlorocyclohexane detected by GC with electron capture detection (Bosch de Basea et al., 2010).

3. Biomarkers of effect

3.1 Biomarkers of effect in aquatic organisms

The use of molecular biomarkers in aquatic organisms is essential to address the broad spectrum of industrial, agricultural, commercial and domestic chemicals that are entering the environment, especially the aquatic environment, and being taken up into the tissues of aquatic organisms. The process of ecological risk assessment is continually developing in ecotoxicological studies to address changing needs and diverse toxicological issues. Risk assessment methods are designed to provide a quantitative estimate of the probability of an adverse effect occurring as a consequence of environmental pollution of a diverse mixture of chemical pollutants, which can also act synergistically (Valavanidis & Vlachogianni, 2010).

Biomarkers of effect and exposure may often be combined into a single biomarker (Barrett et al., 1997). For the purposes of this section, it should be noted that many markers may be used in one or two categories simultaneously.

Among the various types of biomarkers of note in ecotoxicological studies are the following: cytochrome P450 activity (an indicator of the exposure and effect of organic contaminants, such as PAHs, PCBs, and pesticides), the inhibition of AChE activity (a biomarker of the exposure and effect of OP and CAR), metallothionein synthesis in hepatic and other tissues (exposure to the metals Zn, Cu, Cd, Hg, and Fe and some pesticides), and antioxidant enzymes such as superoxide dismutase, catalase, glutathione transferase (exposure to ROS, free radicals, and pollutants causing oxidative stress and lipid peroxidation, such as oxidants, pesticides, and metals).

There are molecular, cellular and whole-animal biomarkers that can be measured in samples of body fluids, cells or tissues. Some biomarkers are specific to a certain set of pollutants, and others change in response to both pollutants and natural factors, causing oxidative stress or adverse effects on biological metabolism. Some biomarkers have prognostic value and can provide an early warning, while others offer specificity, sensitivity or the ability to be applied to a wide range of organisms.

3.1.1 Acetylcholinesterase

AChE is an enzyme responsible for hydrolyzing the neurotransmitter acetylcholine into choline and acetic acid. This enzyme is located in the membranes of vertebrates and invertebrates. The inhibition of AChE is linked directly with the mechanism of toxic action of organophosphorous and carbamade insecticides; the inhibition of this enzyme has also been used to indicate the exposure and effects of other contaminants (cadmium, lead and copper) in fishes, marine bivalves and other organisms (Fulton & Key, 2001; Monserrat et al., 2002; Binelli et al., 2006; Bernal-Hernández et al., 2010; Leite et al., 2010).

Significant depressions of AChE activities in brain and liver tissues of *Oreochromis niloticus* following single and multiple exposures of chlorpyrifos and carbosulfan in the laboratory were reported by Chandrasekara & Pathiratne (2005). Similar results were reported in oysters exposed to dichlorvos (Bernal Hernandez et al., 2010). It has been shown that crude oil in amounts equivalent to sediment concentration inhibits AChE activity in the homogenate of brain fishes (Rodriguez-Fuentes & Gold-Bouchot, 2000). Minier et al. (2000) reported that muscle AChE of flounder from polluted sites with high levels of PAHs was inhibited by 40%. Also, a reduction of 40% of brain AChE was observed in *Mullus barbatus* from three polluted sites in Salento Apulia (Italy) and was related with the presence of great variety of compounds (PAHs, heavy metals and pesticides) in the sediment (Lionetto et al., 2003). Chitmanat et al. (2008) reported low AChE activity in snails (Sinotaia ingallsiana) from Ping River (Thailand), related with the presence of pesticides. Also, other studies have shown seasonal variation of AChE activity in mussel gills, and their changes were related to the periods of pesticide use in the polluted areas (Valavanidis & Vlachogianni, 2010).

3.1.2 Antioxidant enzymes

Biological systems generate endogenous reactive oxygen species (ROS) and other oxidants during their metabolism. Pesticides with redox potential can produce increasing amounts of ROS in marine species in polluted sites. Biological systems are detoxified from ROS by enzymatic and non-enzymatic antioxidant defenses that are ubiquitous in the tissues of most animal species. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, reductase and glutathione S-transferase (GST). The measurement of antioxidant enzymes and lipid peroxidation in aquatic organisms can be used as sensitive biomarkers for the biomonitoring of polluted marine areas containing contaminants, such as pesticides, heavy metals, PAHs, and TCDD, which can generate ROS (Livingstone, 2001; Vlachogianni et al., 2007; Di Giulio & Hinton, 2008).

3.1.3 Heat shock proteins (HSPs)

The exposure of living beings to sub-lethal levels of environmental pollution has been shown to trigger several defense mechanisms at the cellular and molecular levels. There is a cellular accumulation of stress proteins, which mainly act as molecular chaperones (Bauman

et al., 1993; Feder & Hofmann, 1999). Among stress proteins, the HSP70 group has been studied as being regularly over-expressed in response to a wide variety of natural or anthropogenic aggressors (alcohols, oxidative stress, radiations, heavy metals, arsenic, pesticides and others) (Delaney & Klesius, 2004). The role of HSPs during stress is related to a cytoprotective function, as these proteins can act to prevent and repair protein damage (Ananthan et al., 1986). Recently, it was shown that elevated HSP70 is critical in the protection of sea brim cells against chemical-induced apoptosis (Deane et al., 2001). HSP levels have been shown to be modulated in fish cells and tissues upon exposure to an array of stressors (Iwama et al., 1998). Studies performed in low vertebrates are already numerous, and the expression of stress proteins in different fish species in response to various stressors has been investigated by many authors (Iwama et al., 1998, 1999). For instance, several HSPs have been detected after the exposure of various kinds of fish cells to heat shock, arsenate and several metal ions (Misra et al., 1989; Currie et al., 1999, 2000). The accumulation of these HSPs has been linked to the intensity of stress; these proteins have been regarded as a suitable biomarker in assessing reactions of biota to environmental and physiological stressors (Hightower, 1991; Sanders, 1990, 1993).

3.1.4 Metallothioneins (MTs)

Metallothioneins are low-molecular-weight peptides, high in the amino acid cysteine (which contains a thiol group, -SH), that are found mainly in the cytosol, lysosomes and nucleus. MTs are also considered to be stress proteins because they protect cells against excessive metal uptake (Bauman et al., 1993) by virtue of their high proportion of -SH groups, which sequester the metallic ions (Kagi & Schaver, 1988; Klaassen et al., 1999). They are found in many aquatic invertebrates and species of fishes. The overexpression of MTs has been studied in different fish species, and their use as a biomarker for monitoring metal pollution in the environment has been proposed (Carbonell et al., 1998; Hamilton & Mehrle, 1986). Investigations have indicated that simple tissue residue measurements of metals would provide the same information as MTs and would be a better indicator of exposure and effect (Perkins et al., 1996). MT protein determination has a strong correlation with lipid peroxidation in trout chronically exposed to zinc and copper (Farag et al., 1995). Schlenk et al. (1997) examined the effects of low-level arsenic exposure and demonstrated dose-dependent increases in MT expression in channel catfish. In aquatic invertebrates, the development of procedures for the study of MTs is relatively recent. A few studies have shown that digestive glands and gills have the highest concentrations of MTs in aquatic invertebrates (Geffard et al., 2001, 2002; Ceratto et al., 2002; Bernal-Hernández et al., 2010).

3.1.5 CYP1A

Cytochrome P450 monooxygenases (CYPs) are a multi-gene family of enzymes that play a key role in the biotransformation of pollutants, such as dioxins, pesticides, PCBs and PAHs. One of the most common and highly conserved is the CYP1A subfamily. The CYP1A biomarker is widely used as a biomarker of effect both in vertebrates and invertebrates for environmental biomonitoring, especially in marine bivalves and fish (Valavanidis & Vlachogianni, 2010; Di Giulio & Hinton, 2008). The induction of CYP1A is triggered via the cytosolic aryl hydrocarbon (Ah) receptor due to exposure to pollutants, such as polychlorinated biphenyls (PCBs), dioxins, and numerous polycyclic aromatic hydrocarbons (PAHs). Studies have shown relationships between CYP1A expression and reproductive alterations following exposure to PCBs or 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) (Cook

et al., 1997; Teraoka et al., 2003). CYP1A activity is typically measured using the substrate ethoxyresorufin, which is o-deethylated by ethoxyresorufin-O-deethylase (EROD) to a fluorescent product, resorufin, which can be easily measured. Because EROD activities are generally measured using liver homogenates that also tend to accumulate numerous CYP1A substrates, activity may be inhibited by residual substrates or metals (Valavanidis & Vlachogianni, 2010; Di Giulio & Hinton, 2008).

3.2 Biomarkers of effect in humans

Biomarkers of effect in the blood of humans have been related to changes in hemoglobin synthesis upon the exposure to pesticides, as is the case with porphyrins. A large number of organochlorine compounds affect hemoglobin synthesis and result in an accumulation of highly carboxylated porphyrins, which may be detected in the liver, blood, urine or feces (Gil Hernandez, 2000). These have been detected in urine samples from various populations, including workers exposed to hexachlorobenzene and octachlorostyrene (Selden et al., 1999) and a Spanish population environmentally exposed to hexachlorobenzene (Herrero et al., 1999). However, there have been more recent works where there is no alteration in the excretion of urinary porphyrins upon exposure to organochlorines, like the study conducted in a population environmentally exposed to hexachlorobenzene (Sunyer et al., 2002) and in neonates born to exposed mothers (Ozalla et al., 2002).

Regarding the nervous system, it is important to consider that neurochemical measures for the detection of neurotoxicity are limited by the inaccessibility of the target tissue, and hence the identification and characterization of neurotoxicity is dependent on finding parameters in peripheral tissues that reflect the behavior of parameters in the nervous system (Costa & Manzo, 1995). One specific biomarker of neurotoxicity is the inhibition of AChE by anticholinesterase pesticides (OP and CAR). AChE activity is present in many tissues, but its inhibition is usually determined in blood samples (whole blood or plasma) and brain (Gil Hernandez, 2000). This biomarker has been widely used in occupationally exposed populations, such as Egyptian cotton field workers (Farahat et al., 2011), workers occupationally exposed during the manufacture of chlorpyrifos (Garabrant et al., 2009), young children working on Mexican tobacco plantations exposed to OP and CAR pesticides (Gamlin et al., 2007) and farm workers occupationally exposed to agricultural chemicals (Panemangalore et al., 1999), among others.

The effects of certain xenobiotics on the immune system can cause disturbances in normal functioning, decreased resistance to infections or tumors, autoimmune responses and even hypersensitivity reactions (Van Loveren et al., 1995; Kimber, 1995).

Among the assays recommended as biomarkers of immunotoxicity in humans include the following: lymphocyte count, the study of antibody-mediated immunity (Ig in serum), phenotypic analysis of lymphocytes (flow cytometry), the study of cellular immunity, measurements of autoantibodies and markers of inflammatory response, and measurement of nonspecific immunity (Gil Hernandez, 2000). Directly associated with pesticide exposure, adverse effects have been reported in the development of the immune response in two-year-old children living in agricultural areas, who had high levels of Th2, which associated with asthma and wheezing (Duramad et al., 2006).

Another field is the development of adverse effects such as tumors, which are related with xenobiotics and are associated with the aberrant expression of genes encoding proteins involved in cell growth, such as growth factors and oncoproteins. These biomarkers have been studied in plasma or serum samples using ELISA, RIA or immunoblotting and have

also been detected in urine or bronchoalveolar fluid (Gil Hernandez, 2000). In a specific case, it was observed that HER-2/neu oncoprotein is overexpressed in patients with extensive-stage small cell lung cancer and is associated with decreased survival, and it was also observed that pesticide exposure seemed to be related to HER-2/neu overexpression in the study population (Potti et al., 2003). Similarly, an interaction between organophosphate pesticide exposure and PON1 activity on thyroid function in a population of floriculture workers from Mexico was observed (Lacasaña et al., 2010).

Oxidative stress and DNA damage have been proposed as mechanisms linking pesticide exposure to health effects such as cancer and neurological disease (Kisby et al., 2009). In response to oxidative stress, adaptive mechanisms are triggered by protective systems and are commonly quantified in plasma, including the oxidized glutathione (GSSG)/glutathione (GSH) ratio and the activities of glutathione reductase, catalase, superoxide dismutase and peroxidase. Macromolecules that may be affected include lipids, proteins and nucleic acids (Gil Hernandez, 2000). In this regard, a study showed that exposure to OP produces oxidative membrane damage to the erythrocytes of individuals with pathologic complications (Sharma et al., 2010), and a separate study showed an association of oxidative damage with exposure to OP in the blood of horticultural farmers (Atherton et al., 2009). A similar result was observed in a pilot study of pesticide applicators and farm workers working in the fruit orchards of Oregon (Kisby et al., 2009).

More sophisticated techniques allow for the detection of covalent interactions between xenobiotics and proteins and other macromolecules. Many reactive metabolites originating from organic compounds form adducts with proteins or DNA. These can be used as markers of the damage from the exposure to pesticides that causes an increase in the carcinogenic process (Gil Hernandez, 2000). Biological monitoring is done by detection with 32P radiotracers or by immunoassays (with specific antiserum against DNA adducts). Measurements are performed on blood, urine or homogenates of tissues that are obtained from biopsies.

Specifically, adducts have been detected on tyrosine 411 of human albumin exposed to dichlorvos (Li et al., 2010a), and adducts have also been identified at Ser 198 of human BChE upon exposure to carbofuran (Li et al., 2009) and on the DNA-adduct 8-hydroxy-2-deoxy-guanosine (8-OHdG) in the plasma of farm workers (Tope & Panemangalore, 2007), among others.

Pesticides have been considered as potential mutagens because they contain ingredients capable of causing changes in DNA. Therefore, in addition to the determination of adducts, there have been several studies on cytogenetic damage to assess the potential, especially for agricultural workers, for chromosomal aberrations (CA), micronuclei (MN) and sister chromatid exchange (SCE); it has also been possible to determine changes that occur in the kinetics of cell proliferation, which can be observed and evaluated during mitosis. The alkaline single-cell electrophoresis or comet assay (EC) has been designed to assess damage and DNA repair both in vitro and in vivo (Martínez-Valenzuela & Gómez-Arroyo, 2007), but the results are controversial because there are several factors that may cause differences, such as the chemical group to which the pesticide belongs, the technical formulation and active ingredient (which is the product), the type of exposure (chronic or acute), the specific time of exposure for the individual, the manner of contact (direct or indirect), the amount used, the exposure to mixtures, the climate and season of the year, and the person's age, among other factors (Martínez-Valenzuela & Gómez-Arrollo, 2007).

CA, cytological changes, which affect the number or structure of chromosomes that constitute the karyotype of the species, can be observed by light microscopy. These changes correspond to breaks and rearrangements in the same or between different chromosomes (Martínez-Valenzuela & Gómez-Arroyo, 2007).

MN involves the expression at the interface of acentric fragments that do not have centromeres, that are not included in the daughter nuclei during cell division, and that do not interact with mitotic spindle fibers in anaphase; such fragments are surrounded by nuclear membranes and appear as small nuclei. When the damage occurs in the centromere, an imbalance in the distribution of the chromosomes is produced, disturbing the normal kinetics of anaphase and causing envelopment by a nuclear envelope. Assays of this type of damage may be performed using epithelial cells of the urinary bladder and oral and nasal mucosa (Stich & Rosin, 1984; Rosin & Gilbert, 1990) or peripheral blood (Lee et al., 2002; Clare et al., 2006).

SCE occurs during the synthesis phase and represents symmetrical exchanges between homologous loci of replication products, occurring without DNA loss or changes in chromosome morphology (Norppa, 2004). Although not considered to be mutations, sister chromatid exchanges have been noted to increase in frequency when cells are exposed to known mutagenic and carcinogenic agents. Tests of such disturbances are used in the biological monitoring of individuals exposed to potential or known genotoxic agents (Lambert et al., 1982; Cavallo et al., 2006).

Known as single-cell alkaline electrophoresis, EC is a fast, simple, visual and sensitive biomarker used to measure and analyze breaks in DNA. This assay detects intracellular differences and damage to the repair processes of cells (Speit & Hartmann, 2006). The comet assay consists of quantifying the damage induced in the DNA of cells that are embedded in agarose, lysed and then subjected to electrophoresis in alkaline pH, which ensures that fragments of chromosomes are directed toward the anode and are revealed as the tail of a comet upon staining with a fluorescent dye (Tice et al., 2000). The extent of DNA migration depends on the number of breaks produced by the agent in question (Garaj-Vrhovac & Zeljezic, 2001), so each cell has the appearance of a comet with a head and tail under bright fluorescence, while undamaged cells appear as intact nuclei without tails (Møller, 2006).

Cytogenetic biomarkers have been widely used in populations occupationally exposed to pesticides, including European farmers (Pastor et al., 2003), female workers exposed to pesticides in banana plantations in Costa Rica (Ramírez & Cuenca, 2002), Mexican retailers (Rojas-García et al., 2011), workers involved in the pesticide manufacturing industry in Pakistan (Bhalli et al., 2006), domestic users of OP (Lieberman et al., 1998), and populations residing in pesticide-contaminated regions in Göksu Delta (Ergene et al., 2007), among many others.

However, it is important to consider that studies of pesticide exposure and genotoxic effects must take into account the reliability of damage from exposure, the strength of the studies, the similarity of the control group and the protocols used for genotoxicity (Bull et al., 2006).

4. Susceptibility biomarkers

Phenotypic and genotypic variation between individuals is a fundamental characteristic of living beings. With the revealing of the human genome, more "susceptibility" genes will be discovered, and it is likely that the etiology of many diseases or health outcomes will be shown to be related to a combination of genetics and environment. Simple blood tests may

ultimately be developed that allow an individual to learn whether he or she may be particularly susceptible to specific environmental pollutants (Klaassen, 2008). In the case of pesticides, there is growing recognition that genetic factors can account for individual susceptibility to a range of responses.

Pesticide adverse effects result from the complex interactions between internal (genes, age, sex, species, pathological and physiological status) and external factors (temperature, diet, lifestyle and others). The adverse health effects of pesticides are in most cases related with the toxicokinetics of those compounds; variabilities in the absorption, distribution, biotransformation and excretion of pesticides may play essential roles in the modulation of the internal dose and the effects (Figure 1).

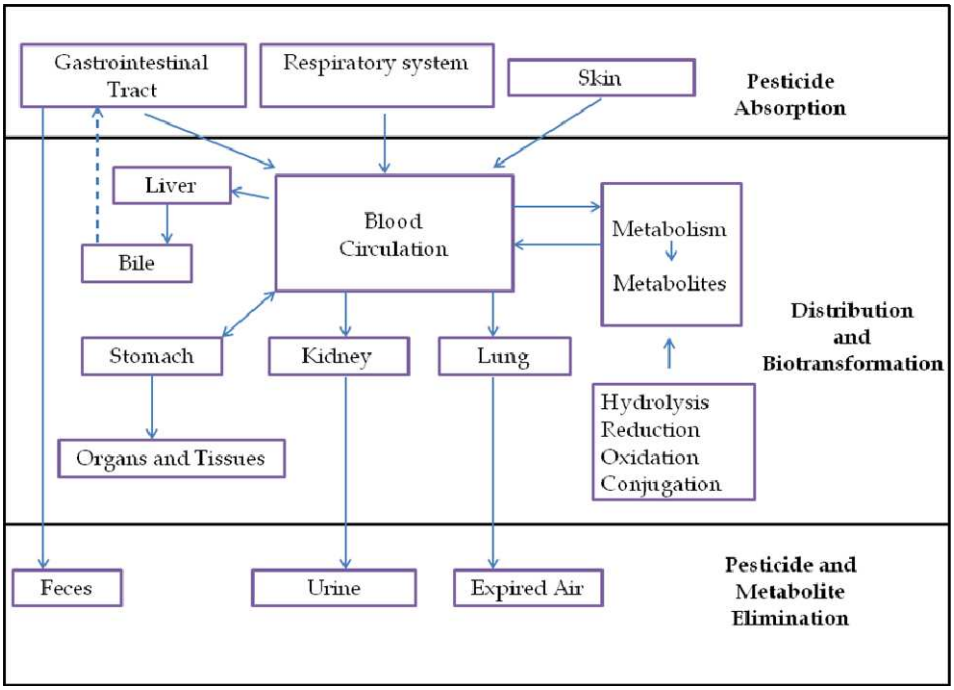

Fig. 1. General pesticide pathways in an organism

Some pesticides are bioactivated by metabolic enzymes in organisms and are converted into toxic compounds. In addition, there are also enzymes that participate in the detoxification of pesticides. It is important to evaluate the bioactivation/inactivation ratio in individuals to produce a more complete scenario of pesticide susceptibility.

In this regard, pesticides are metabolized by a variety of cytosolic and microsomal enzymes. Biotransformation of pesticides is a fast process that involves different families of enzymes, such as hydrolases, oxidases, reductases, and conjugation enzymes. There is evidence that the genes coding for the different enzymes that biotransform pesticides have genetic variations in human populations. Changes in the biotransformation ability of these compounds can have an impact on the toxicokinetics and thus the toxicity of these pollutants.

4.1 Cytochrome P450 (CYP450)

CYP450 is a family of hemoproteins that catalyze monooxygenation reactions (Santiago et al., 2002); as a result of these reactions, P450 accelerates the body's elimination of many drugs and toxic compounds, but it is also responsible for the activation of toxins or pre-carcinogens (Donato, 2004). All known P450s are named according to common criteria and are grouped into families and subfamilies based on similarities in the encoding DNA sequences (Donato, 2004). In humans, 18 families and 43 subfamilies of CYP450 have been identified (Nelson, 2002). Families 1, 2 and 3 are made up of enzymes responsible for the biotransformation of xenobiotics, while other families are involved in the biosynthesis and metabolism of endogenous compounds (Donato, 2004). The P450s are widely distributed throughout the body, but the liver is the organ with the highest expression of these enzymes. Its expression is regulated by genetic, pathophysiological and environmental factors (Donato, 2004).

Of the cytochrome P450 members, CYP2D6 is the one with the greatest genetic influence (Espíritu, 2008). The gene that encodes it is located near two pseudogenes on chromosome 22q13.1, CYP2D7P and CYP2D8P (Grimán et al., 2009). CYP2D6 is involved in the metabolism of many drugs, particularly those that work in the central nervous system and cardiovascular system, but it also catalyzes the oxidative biotransformation of organophosphorus pesticides such as parathion and diazinon (Schaeffeler et al. 2003). CYP2D6 has variations in its gene sequence, called polymorphisms, that may or may not change its amino acid sequence. For each CYP isoenzyme, there are several genetic polymorphisms, some of which are critical in the metabolism of drugs and environmental pollutants such as pesticides (Espíritu, 2008). The diversity of polymorphisms in this gene produces four phenotypes known as poor (PM), intermediate (MI), rapid or extensive (EM) and ultrarapid metabolizers (UM); these phenotypes are associated with variable responses to drugs, adverse reactions to drugs upon increasing the concentrations of drugs in the PM, or treatment failure as a result of the degradation of drugs in UM (Schaeffeler et al., 2003). In the case of pesticides, it is expected that these phenotypes are related to differences in the bioactivation abilities and differences in the toxicities of these xenobiotics. There are about 20 polymorphisms related to the phenotype MP; 95% correspond to the alleles * 3, * 4, * 5 and * 6, with the most frequent allele as * 4, characterized by a base substitution G1934A in the splicing site between introns and exons three and four; a truncated protein results from this mutation (Grimán et al., 2009). CYP1A2 and 2B6 have also been reported as possible metabolic biomarkers of susceptibility to OP-induced toxic effects at actual human exposure levels (Buratti et al., 2005).

Of note, organochlorine pesticides (OCP) and polymorphisms of xenobiotic metabolizing enzymes are reported to be associated with a possible risk of prostate cancer. OCPs are endocrine disruptors (ED) that may act by disrupting the physiologic function of endogenous hormones and therefore possibly increase prostate cancer risk. CYP1A1 metabolizes several carcinogens and estrogens, and polymorphisms of this gene have been reported to be associated with prostate cancer risk. Kumar et al. (2010) studied 70 newly diagnosed prostate cancer patients and 61 age-matched healthy male controls. OCP levels in blood were determined, and CYP1A1 polymorphisms were analyzed. Significantly higher levels of β-HCH, γ-HCH and p,p'-DDE were found in cases as compared to controls (p-values=0.04, 0.008, and 0.01, respectively). Higher levels of γ-HCH were observed in advanced stages of prostate cancer cases (<or=T(2) vs. >or=T(3)) (p=0.04). Dieldrin was found to be significantly higher in cases with initial stages (p=0.03). However, there was no

observed correlation between prostate cancer and CYP1A1 polymorphisms. Hence, higher level of OCPs, especially β-HCH, γ-HCH and p,p'-DDE, might be associated with prostate cancer risk.

4.2 Glutathione S-transferase (GSTs)

The glutathione S transferases are classified into eight families (alpha, kappa, mu, pi, sigma, theta, zeta and omega) based on the amino acid sequence, immunogenic properties and physiological role. These enzymes may be localized in the cytoplasm or in the endoplasmic reticulum of cells and are present in almost all tissues, but their greatest expressions occur in liver, kidney, intestine, testis and lung (Klaassen, 2008). GSTs catalyze the nucleophilic conjugation of different biologically active and potentially carcinogenic compounds that can be further biotransformed to mercapturic acids (Ortiz et al., 2001; Oude et al., 1998). Among the GST substrates are a variety of pesticides, and GST seems to play an important role in the elimination and hence in the detoxification of these compounds. GSTs include a superfamily of highly polymorphic genes (Stanulla et al., 2000). In humans, polymorphisms of GST associated with a decreased activity (GTSM1, GSTM3, GSTM4, GSTP1, GSTT1 and GSTZ1) have been identified (Fernández, 2005).

The most important polymorphisms are those on the locus GTSM1, which has four variant alleles: GSTM1 * A, GSTM1 * B, GSTM1 * 0 o C and GSTM1 null. The first three have no apparent differences in catalytic activity. The variant null, or 0, is a partial deletion of the gene that leads to a total loss of enzyme activity (Oude et al., 1998). This null polymorphism is present in 30-60% in the general population and has been associated with a risk of developing lung cancer in Asian, Caucasian and Latino populations (Ortega, 2007; Fernandez, 2005). Also, several studies have demonstrated a direct association of this polymorphism with increased risks of bladder, gastric, colorectal and skin cancers (Lan et al., 2000; Stucker et al., 2002).

In a study conducted on agricultural workers of the Punjab region of northwestern India, the GSTT1 gene deletion and simultaneous deletions of GSTM1 and GSTT1 genes were related with increasing DNA damage evaluated using an alkaline comet assay (Abhishek et al., 2010).

4.3 Human serum paraoxonases (PONs)

Human serum paraoxonases are a multigene family comprised of PON1, PON2 and PON3. Among the PONs, PON1 (EC 3.1.8.1) is the most studied family member. PON1 is a 355-amino-acid calcium-dependent esterase predominantly synthesized in the liver and closely associated with high-density lipoproteins (HDL). Even though PON1 has no known physiological substrate and no clear biological function, it is known that PON1 is capable of hydrolyzing certain toxic metabolites of OP, such as paraoxon, diazoxon, and chlorpyrifos oxon, as well as nerve agents, such as soman and sarin. This hydrolyzation significantly influences the detoxification of the toxic compounds and hence can modify their toxicities. Besides being capable of hydrolyzing OP, PON1 has been shown to hydrolyze phenyl acetate, an aromatic carboxyl ester, and hence is involved in the metabolism of drugs and xenobiotics. Furthermore, PON1 hydrolyzes some naturally occurring lactone metabolites and estrogen esters.

The concentration of PON1 in human plasma varies among individuals. PON1 activity levels are determined by a combination of complex genetic interactions and environmental-

dietary factors, leading to a 40-fold variation in PON1 in single individuals. In addition, the PON1 gene shows several polymorphisms in the promoter and coding regions, which explain, at least in part, the large variations in activity and concentration among individuals.

4.3.1 Polymorphisms in the coding region

Two common polymorphisms in the coding region of PON1 have been reported, at positions 55 and 192. The polymorphism at position 55 (Leu/Met) has been related with different PON1 activities, with the 55M isoenzyme having lower enzymatic activity than the 55L isoenzyme (Blatter-Garin et al., 1997). It has been proposed that this is due in part to linkage disequilibrium with the -108C allele (Brophy et al., 2001) and also to an increase in the stability of the 55L isoenzyme (Leviev et al., 2000). More attention has been paid to the 192 polymorphism because the two allozymes differ considerably in their affinities and catalytic activities with a number of substrates (Draganov et al., 2004). This polymorphism (192 Glu/Arg) has two isoforms that hydrolyze phenylacetate at similar rates (La Du et al., 1986), but their paraoxon and diazoxon hydrolysis rates are different. Linkage disequilibrium between PON1 55 and PON1 192 has been reported (Blatter-Garin et al., 1997; Brophy et al., 2000; Rojas-García et al., 2005).

It has been suggested that serum paraoxonase is mostly involved in the detoxification of a variety of OP compounds in mammalian species. One study showed that species lacking paraoxonase are more susceptible to OP toxicity (Mackness et al., 1998). This finding supports the hypothesis that serum paraoxonase is the main enzyme responsible for the deactivation of OP compounds, thereby reducing the risk to the toxic effect of the corresponding oxons.

Experiments have investigated the toxicity of OP in PON1 knockout (PON1-/-) mice, which have no serum paraoxonase or diazoxonase activity and a very low activity toward chlorpyrifos oxon. PON1 knockout mice have dramatically increased sensitivities to chlorpyrifos oxon and diazoxon and slightly increased sensitivities to chlorpyrifos and diazinon, but they do not show increased sensitivity to paraoxon (Costa et al., 2003). Studies in animals show that injection of partially purified PON1 into rats increases their resistance to paraoxon. Additionally, injection of purified rabbit PON1 into mice 4 h prior to exposure to chlorpyrifos dramatically increases their resistance to chlorpyrifos and its oxon. These experiments support the hypothesis that high levels of plasma paraoxonase could protect against exposure to chlorpyrifos (Furlong et al., 2005). Further studies have investigated whether the administration of exogenous PON1 restores plasma PON1 levels in PON1-/- mice and whether these PON1 levels in plasma provide protection against OP toxicity. Human PON1Q192 or PON1R192 were injected intravenously into PON1-/- mice, and the effects of OP on brain and diaphragm AChE were determined. Both isoforms (PON1R192 and PON1Q192) were protective to chlorpyrifos oxon and diazoxon, but neither human PON1 isoform protected against the toxicity of paraoxon (Costa et al., 2003).

Some studies in the literature evaluate the role of PON1 as a susceptibility biomarker to adverse effects of OP. Most of them analyze the PON1 polymorphisms and their relationship with the neurological effects caused by OP. In this regard, Haley et al. (1999) reported that ill veterans with neurological symptom complexes were more likely to have the R allele than to be homozygous for Q. Additionally, Sirivarasai et al. (2007) found a relationship between the PON1 polymorphism and cholinesterase activities in an OP-exposed population. In agreement with this, there are reports showing that PON1 genotypes

are associated with exposure-related changes in paraoxonase, arylesterase and acetylcholinesterase activities and with abnormal electroencephalography patterns at sub-threshold pesticide exposure (Browne et al., 2006). Similarly, Mackness et al. (2003) investigated the relationship between PON1 genetic polymorphisms and PON1 activity in farmers who reported chronic symptoms related to OP exposure, concretely in sheep dipping (cases) and controls. Individuals showing toxic effects were found to be more likely to have the R192 and L55 alleles than the controls, and the combination of R and L genotypes was associated with lower PON1 activity toward diazoxon. Additionally, the farmers reporting chronic symptoms due to OP exposure had a higher proportion of the PON1 192R polymorphism, which is associated with lower rates of diazoxon hydrolysis, as compared to controls. Their symptoms may be explained by a lower ability to detoxify diazoxon.

The role of PON1 genotypes in OP toxicity has also been investigated in OP-poisoned patients. For this purpose, Akgür et al. (2003) evaluated the effect of PON1 on the outcome of acute OP intoxication and the effect of this on PON1. The frequency of the PON192Q allele was significantly higher in patients than controls, which suggests that differences in PON1 activity and the PON1 55 and 192 polymorphisms are important risk factors in the susceptibility to acute OP poisoning. In addition, in another work conducted on acute OP insecticide poisoning cases, a correlation between the stimulation of PON1 and butyrylcholinesterase activity was found, but this correlation was lower than that in cases with chronic exposure to OP insecticides. The authors suggest that in both chronic and acute OP exposures, both PON1 levels and phenotypes must be taken into consideration.

The effects of PON1 genotypes on male reproductive outcomes related to OP exposure have been poorly investigated. In one study, Padungtod et al. (1999) studied the allele frequency of PON1 192 and its relationship with semen quality and hormone profile in Chinese pesticide-factory workers and in controls. Both unexposed 192QQ and exposed 192QR showed significantly lower sperm concentrations than the reference group. In addition, exposed individuals carrying at least one R allele had significantly higher serum LH (luteinizing hormone) levels than the control group. Similarly, a cross-sectional study of farmers with Mayan ascendancy from southeastern Mexico chronically exposed to pesticides (mostly OP) was performed by Pérez-Herrera et al. (2008). Exposure to OP was associated with *in situ* nick-translation-positive cells and sperm viability in homozygote 192RR subjects. Furthermore, dose-effect relationships were observed between OP exposure for three months before sampling and both sperm quality parameters and nick-translation-positive cells in 192RR farmers. The authors suggest that PON1Q192R polymorphisms could modulate the OP-mediated toxicity on spermatogenic cells.

In a study conducted by Singh et al. (2011), several related aspects were evaluated: (a) the prevalence of two common PON1 polymorphisms, (b) the activity of PON1 and acetylcholinesterase enzymes, and (c) the influence of PON1 genotypes and phenotypes variation on DNA damage in workers exposed to OP. A total of 230 subjects were examined, including 115 workers exposed to OP and an equal number of normal healthy controls. The results revealed that PON1 activities toward paraoxon (179.19±39.36 *vs*. 241.52±42.32 nmol/min/ml in controls) and phenylacetate (112.74±17.37 *vs*. 134.28±25.49 µmol/min/ml in controls) were significantly lower in workers than in control subjects ($p<0.001$). No significant differences were observed in the distributions of genotypes and allelic frequencies of PON1(192)QR (Gln/Arg) and PON1(55)LM (Leu/Met) in workers and control subjects ($p>0.05$). The PON1 activity toward paraoxonase was found to be

significantly higher in the R/R (Arg/Arg) genotype than the Q/R (Gln/Arg) genotype and was lowest in Q/Q (Gln/Gln) genotype in both workers and control subjects ($p<0.001$). For PON1(55)LM (Leu/Met), PON1 activity toward paraoxonase was observed to be higher in individuals with the L/L (Leu/Leu) genotype and was lowest in individuals with the M/M (Met/Met) genotype in both groups ($p<0.001$). No influence of the PON1 genotype or phenotype was seen on the activity of acetylcholinesterase or arylesterase. The DNA damage was observed to be significantly higher in workers than in control subjects ($p<0.05$). Furthermore, the individuals who showed least paraoxonase activity, i.e., those with (Q/Q [Gln/Gln] and M/M [Met/Met]) genotypes, showed significantly higher DNA damage compared to other isoforms in workers exposed to OP ($p<0.05$). These results indicate that individuals with PON1 Q/Q and M/M genotypes are more susceptible to genotoxicity. The study concluded that there were wide variations in enzyme activities and DNA damage due to polymorphisms in the PON1 gene that might have important roles in the identification of individual risk factors in workers occupationally exposed to OP.
In addition, there have been studies where OP pesticides and adverse pregnancy outcomes were analyzed. In this regard, Wolff et al. (2007) measured biomarkers of maternal exposure to DDE, PCB, and OP metabolites in pregnancy among exposed mothers, as well as maternal paraoxonase (PON1), BChE, and PON1Q192R gene variants. They found that infant birth lengths were shorter for mothers with the PON192RR genotype compared with PON192QQ, and head circumference was inversely associated with maternal PON1 activity. A relationship between prenatal environmental biomarkers and birth outcomes modulated by PON1 and maternal weight was suggested.

4.3.2 Studies with two or more enzymes as susceptibility biomarkers

Hernández et al. (2005) conducted a study in 135 pesticide applicators (sprayers), and the authors investigated changes in erythrocyte delta-aminolevulinic acid dehydratase (ALA-D) after exposure to different pesticides, including OP and paraquat. AChE was used as a reference biomarker. The effects of the combined polymorphisms of enzymes involved in the detoxification of pesticides (PON1, benzoylcholinesterase (BeChE), and glutathione S-transferase (GSTM1 and GSTT1)) on the levels of the target erythrocyte enzymes were also studied as biomarkers of individual susceptibility. Sprayers presented significantly lower levels of ALA-D and AChE than controls (41.3% and 14.5%, respectively) at the high exposure period. When all biomarkers of individual susceptibility to pesticides were considered at the same time, the GSTT1 null allele determined higher ALA-D and AChE activities at the period of high exposure to pesticides. The PON1 R allele in turn determined lower AChE activity at the low exposure period. Null genotypes for both GST subclasses (GSTM1 and GSTT1) were found to be unique independent predictors of pesticide-related symptomology. Interestingly, sprayers were consistently underrepresented among carriers of "unfavorable" BeChE variants. The conclusions of the study were that ALA-D appears to be an important biological indicator of pesticide exposure and that PON1 and GSTT1 are relevant determinants of susceptibility to chronic pesticide poisoning.
A study conducted by Tsatsakis et al. (2009) investigated the correlation of CYP1A1 and PON1 enzymes with the incidence of various medical examination findings in a Greek rural population professionally exposed to a variety of pesticides. The medical history of 492 individuals, randomly selected from the total population of 42,000, was acquired by interviews, and their genotypes were determined for CYP1A1*2A, PON1 M/L and PON1 Q/R polymorphisms. The assessment of the population's pesticide exposure was verified by

analytical methods. Analysis of the genetic data showed that the allele frequencies of PON1 R, M and CYP1A1*2A alleles were 0.243, 0.39 and 0.107, respectively. The CYP1A1*2A polymorphism was found to have a significant association with chronic obstructive pneumonopathy (p=0.045), peripheral circulatory problems (trend p=0.042), arteritis (p=0.022), allergies (trend p=0.046), hemorrhoids (trend p=0.026), allergic dermatitis (p=0.0016) and miscarriages (p=0.012). The PON1 Q/R polymorphism was found to have a significant association with hypertension (p=0.046) and chronic constipation (p=0.028), whereas the L/M polymorphism was associated with diabetes (p=0.036), arteritis (trend p=0.022) and hemorrhoids (trend p=0.027). These results demonstrate an association between the CYP1A1/PON1 polymorphisms and several medical examination findings, indicating the possible involvement of the human detoxification system in the health effects of a rural population exposed professionally to pesticides.

Greater appreciation of the mechanisms and extent of individual variation in the susceptibility among humans can improve the protection of susceptible populations and better relate findings in animals to the characterization of risk in humans.

By definition, biomarkers do not directly provide information concerning impacts on the higher levels of organization that ecotoxicology ultimately endeavors to discern. Nevertheless, biomarkers can provide important ancillary tools for revealing contaminant exposure and potential impacts of ecological importance.

The development and use of biomarkers in ecotoxicology is motivated by several factors. These factors include the inherent instabilities of many contaminants (which complicate measures of exposure by direct tissue residue analysis), the relative biological sensitivities of many biomarkers, and the chemical specificity of some biomarkers on underlying mechanisms of toxin action. Additionally, the variables associated with these levels are often relatively insensitive to chemicals, such as some pesticides and other stressors, take long periods of time to manifest, and have difficult or imprecise methods of analysis. Thus, biomarkers can provide sensitive early warning signals of incipient ecological damage. However, biomarkers do not provide adequate standard data in the context of the ecological assessment of contaminant effects. At this time and for the foreseeable future, such assessments generally involve a "weight of evidence approach", coalescing information obtained from analyses, toxicity tests, biomarkers, and ecological indicators, which are sometimes referred to as bioindicators.

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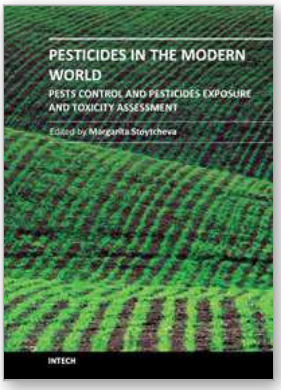

Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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