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## Biological Markers of Human Exposure to Pesticides

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### 1. Introduction

Pesticides are used extensively throughout the world and, in recent years, their use has increased considerably. Large amounts of these chemicals are released into the environment and many of them affect non-target organisms, being a potential hazard to human health. Pesticide exposure is ubiquitous, due not only to agricultural pesticide use and contamination of foods, but also to the extensive use of these products in and around residences. Hence, all people are inevitably exposed to these toxics through environmental contamination or occupational use. The general population is exposed to the residue of pesticides, including physical and biological degradation products in air, water and food. Occupational exposure occurring at all stages of pesticide formulation, manufacture and application involves exposure to complex mixtures of different types of these chemicals.

Pesticides are responsible for several adverse effects on human health, and they represent a potential risk to human. Several studies revealed that the risk of neurodegenerative diseases, particularly Parkinson's and Alzheimer disease, as well as the increase in endocrine, immune and neuropsychological disorders are among the harmful effects of these compounds on human health (Stephens et al., 1995; Parron et al., 1996; Mathur et al., 2002; Baldi et al., 2003; Hernandez et al., 2003; Salvi et al., 2003; Kamel & Hoppin, 2004). Other reports indicated that pesticides possess a potential genotoxicity in occupationally exposed populations and they induced some types of cancers (Anwar, 1997; Mathur et al., 2002; Hernandez et al., 2003; Clary & Ritz, 2003; Rusiecki et al., 2004; Bolognesi, 2003).

Defending against such toxic compounds requires sensitive and specific detection of them and their biological effects. Thus, the biomonitoring is a useful tool for assessing exposure to pesticides and for the evaluation of potential health risks, because of the various routes of exposure involved and the possible combination of occupational and non-occupational exposures. The biological monitoring methods are becoming available for a greater variety and number of pesticides, and biomonitoring data are being used to validate questionnaires as meaningful indicators of exposure. However, these biomonitoring data are difficult to interpret, and currently there are no guidelines for their interpretation. The lack of human pharmacokinetic data for many pesticides impedes efforts to estimate external exposure or target organ dose. A major problem in interpreting biomonitoring studies is estimating the degree of exposure (Bolognesi, 2003; Remor et al., 2009). Moreover, the relationship between internal exposure and health effects was often unknown.

In recent years, increasing attention has been given to the development of biomarkers of human exposures to pesticides. These biological markers are used to detect the effects of pesticides before adverse clinical health effect occur. In human, biomarkers must be present in easily and ethically obtainable tissues such as blood or urine. Biomarkers are usually divided in three categories: biomarkers of exposure, of effect and of susceptibility.

Biological markers of exposure can be used to confirm and assess the exposure of populations to a particular substance. These biomarkers provide information on the potential or external dose, internal or absorbed dose and biologically effective dose of pesticide, and they can predict a change in risk of that disease, but it cannot predict a toxic effect (Benford et al., 2000). However, biomarkers of effect can be used to document either preclinical alterations or adverse health effects elicited by the external exposure and the absorption of a compound. These biomarkers might reflect an early stage in the development of a disease, and therefore may be predictive of eventual disease (Benford et al., 2000). Genotypes responsible for interindividual differences in enzymes involved bioactivation and detoxification of pesticides are recognised as biomarkers of susceptibility to pesticide toxicity. The individual susceptibility, caused by polymorphic key enzymes, plays a critical role in the assessment of exposure to pesticides.

This review will focus and describe selected aspects of the biomarkers used for the assessment of human exposure to pesticides.

## 2. Biomarkers

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 (WHO, 1993).

Biological markers for monitoring pesticide exposures are typically divided into three broad categories: Biomarkers of exposure, effect and biomarkers of susceptibility (Figure 1).

### 2.1 Biomarkers of exposure

The biomarkers of exposure constitute 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 (WHO, 1993).

Knowledge of exposure levels is a first step in risk-evaluation process, and can be acquired by measuring the dose of pesticide entering the body. Hence, biological indicator of exposure or dose is the measurement of chemical agents or their metabolites either in tissues, secreta, excreta, exhaled air, or any combination of them, in order to evaluate exposure and health risk compared to an appropriate reference (Aprea et al., 2002). These biomarkers can predict a change in risk of that disease, but it cannot predict a toxic effect (Benford et al., 2000).

The biomarkers of exposure can be divided into three groups: potential dose or external dose, internal or absorbed dose, and biologically effective dose.

Because human exposure to pesticides is multi-media and multi-route and varies with the usage of pesticides, environmental monitoring of exposure which determines the potential dose, must account for all media and routes in order to accurately calculate individual exposures.

Biomarkers of internal dose integrate all pathways of exposure by estimating the amount of pesticide that is absorbed into the body via measurements of the pesticide, its metabolite, or its reaction product in biological media.

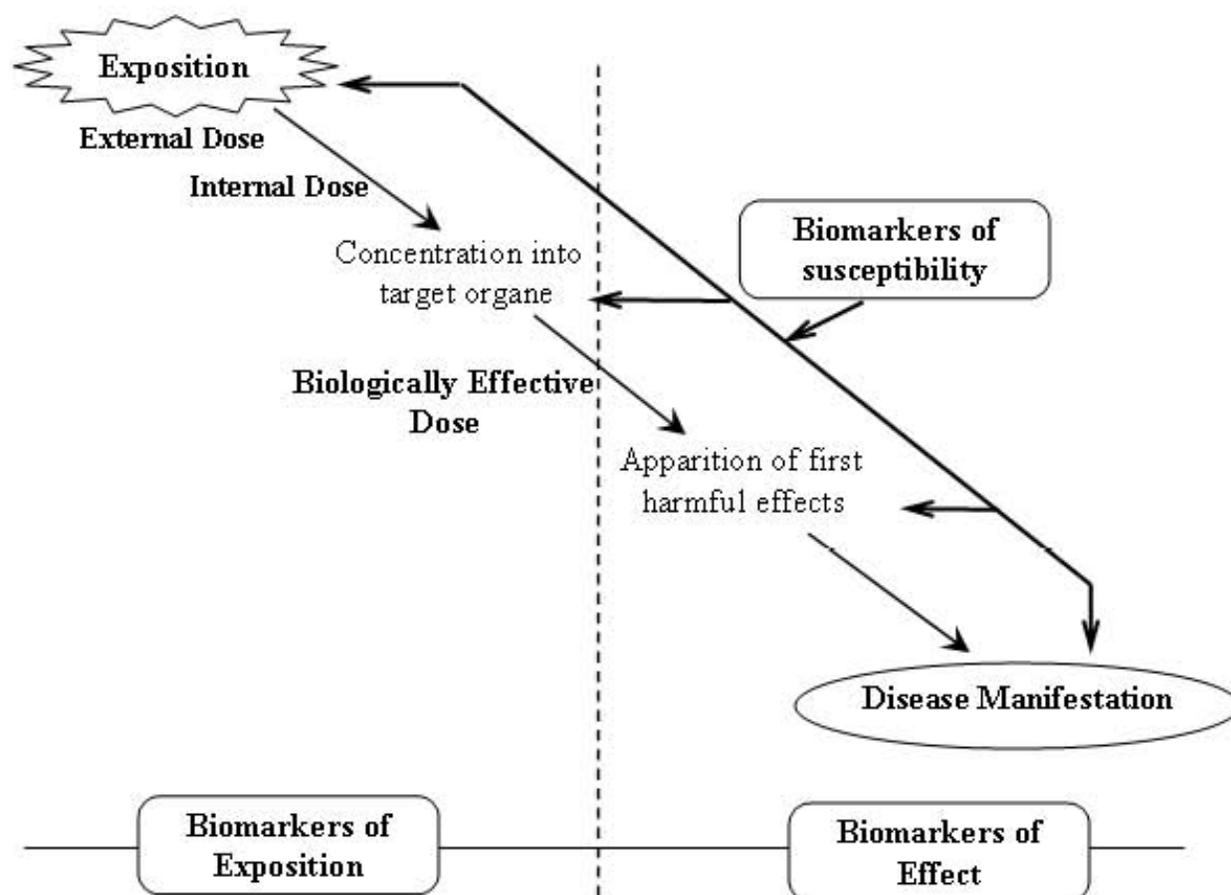


Fig. 1. Categories of biological markers of toxicity

The biologically effective dose is the amount of a toxicant that has interacted with a target site and altered a physiological function (Aprea et al., 2002).

Biological indicators of exposure can usually be measured by different analytical methods. These methods all use some form of chromatography, but the detection systems range from simple UV absorbance detection to sophisticated mass spectrometric analyses (Frias et al., 2001; Smith et al., 2002; Barr et al., 2002; Aprea et al., 2002; Bouwman et al., 2006; Russo et al., 2002; Lacassie et al., 2001a). They have been used to measure pesticides and/or their metabolites in a variety of matrices including urine, serum, breast milk, adipose tissue and postpartum meconium.

This followed section will focus the main biological indicators of exposure to insecticides.

## 2.1.1 Intact compounds

### 2.1.1.1 Organochlorine compounds

Organochlorine (OC) pesticides are a broad class of pesticides that were widely used as insecticides in the 1950 and 1960s. Their use was subsequently discontinued in many countries due to persistent contamination of the environment. Determination of intact OC compounds is a valuable method to monitor short- and long-term exposures to these pesticides (Table1). Exposure to OC pesticides has been investigated by several studies, in occupationally exposed subjects and in the general population by measuring intact compounds in blood, urine, adipose tissue and human milk (Frias et al., 2001; Barr et al.,

2002; Heudorf et al., 2003; Thomas et al., 2006; Bouwman et al., 2006; Ennaceur et al., 2008; Tsatsakis et al., 2009; Turci et al., 2010). Dichlorodiphenyltrichloroethane (DDT), dieldrin, hexachlorocyclohexane isomers (HCHs) and heptachlor-epoxide are the pesticide residues most frequently found in biological fluids of the general population.

#### 2.1.1.2 Organophosphorous compounds

Measurement of unchanged organophosphorous (OP) pesticides in blood, and/or urine, or in gastric content has often been performed to confirm exposure in acute poisoning cases (Maroni et al., 2000a; Lacassie et al., 2001a,b; Aprea et al., 2002; Inoue et al., 2007). In fatalities, unchanged compounds may be measured in central nervous system and other tissues (Aprea et al., 2002). However, this method is unfavourable for biological monitoring of occupational exposure. At the dose levels usually found in exposed workers, OP compounds disappear quickly from blood and are rapidly excreted in urine at concentrations usually too low to be detected (Maroni et al., 2000a).

#### 2.1.1.3 Carbamate compounds

Measurement of unmodified carbamate (CB) pesticides in blood and/or urine has often been performed to confirm exposure in acute poisoning cases (Table1), in order to identify the agent responsible for the intoxication (Lee et al., 1999; Lacassie et al., 2001b). In fatal cases, intact compounds may be measured in various organs. In cases of occupational exposure, unmodified compounds is rarely measured since the metabolic pathway of these substances is very complex, and brings forth a number of polarized molecules, which are more water-soluble than parent compounds (Maroni et al., 2000b; Aprea et al., 2002).

#### 2.1.1.4 Pyrethroids compounds

Synthetic pyrethroids are a group of insecticides largely used in agriculture and public health because of their relatively low toxicity to man and mammalian species at the usual application rates, and because of their short environmental persistence. Occupational exposure to pyrethroids may be assessed by measuring intact compounds or their metabolites in urine. As a result of their rapid metabolism, determination of blood concentrations can only be used to reveal recent high level exposures (Table 1). Because of their rapid elimination, unmodified compounds are less sensitive indicators of exposure than metabolites, although certainly more specific (Aprea et al., 2002). In field workers exposed through the dermal route, urinary excretion of intact compounds and metabolites peaked 36 hours after exposure.

Table1 summarized some examples of intact insecticide compounds measured in human biological samples.

Pesticides	Exposure characteristics	Sample	Analytical methods	References
<b>Organochlorine compounds</b>				
$\alpha$ - et $\beta$ -endosulfan	Acute intoxication cases	Serum	GC/MS*	Lacassie et al., 2001b
Lindane, Aldrin, Vinclozolin, p,p'-DDT, o,p'-DDT	Women living agricultural area	Serum	GC/ECD* GC/MS/MS*	Frias et al., 2001
$\beta$ -HCH, p,p'-DDT, o,p'-DDT	General population	Serum	GC/MS*	Turci et al., 2006

Pesticides	Exposure characteristics	Sample	Analytical methods	References
$\beta$ -HCH, p,p'-DDT	General adult population	Serum	GC/MS*	Thomas et al., 2006
DDT, HCH, dieldrin	General population	Breast milk	GC/ECD*	Ennaceur et al., 2008
$\alpha$ -HCH, p,p'-DDT, lindane	Population professionally exposed to pesticides	Serum	GC/MS*	Tsatsakis et al., 2009
<b>Organophosphorous compounds</b>				
Parathion-ethyl	Poisoning cases	Serum	GC/MS*	Lacassie et al., 2001a,b
Fenitrothion, diazinon, acephate	Poisoning cases	Serum	LC/MS*	Inoue et al., 2007
Diazinon, malathion, chlorpyrifos	Population professionally exposed to pesticides	Serum	GC/MS*	Tsatsakis et al., 2009
18 OP detected 20 OP detected	Patients with various causes of death (without pathologies or with cancer pathologies)	Kidney, Liver, adipose tissues	GC/MS*	Russo et al., 2002
<b>Carbamates compounds</b>				
Furathiocarb	Fatal poisoning case	Gastric contents	GC/MS*	Lee et al., 1999
Carbofuran, aldicarb	Poisoning cases	Serum	LC/MS*	Lacassie et al., 2001b
Carbofuran Carbendazime	Agricultural workers	Serum	LC/MS/MS*	Araoud et al., 2010
<b>Pyrethroid compounds</b>				
Bifenthrin	Poisoning cases	Serum	GC/MS*	Lacassie et al., 2001b
Cyfluthrin, Cypermethrin, Permethrin, Deltamethrin	General population	Breast milk	GC/ECD* GC/MS*	Bouwman et al., 2006

\*: GC/MS: gas chromatography coupled to mass spectrometry; GC/ECD: gas chromatography coupled to electron-capture detector;

GC/MS/MS: gas chromatography tandem mass spectrometry; LC/MS/MS: liquid chromatography tandem mass spectrometry.

Table 1. Examples of intact insecticide compounds measured in human biological samples

## 2.1.2 Pesticide metabolites

### 2.1.2.1 OC metabolites

Biological monitoring of OC can be carried out by determination of their metabolites in biological samples (Frias et al., 2001; Aprea et al., 2002; Bouwman et al., 2006; Ennaceur et al., 2008; Tsatsakis et al., 2009). Table 2 lists the main OC pesticides and their metabolites, for which data on the use of biological indicators of internal dose have been found in literature.

Compounds	Metabolites	Biological samples
Dichlorodiphenyl trichloroethane (DDT)	Mainly p,p'-dichlorodiphenyl dichloroethylene (p,p'-DDE)	Adipose tissue, blood, breast milk, urine
Aldrin	Dieldin	Blood, serum, fatty tissue, milk
Endrin	Anti-12-hydroxy-endrin	Urine
Heptachlore	Heptachlore-epoxide	Milk, serum, adipose tissue, urine

Table 2. Metabolites of main OC pesticides used for biological monitoring of human exposure to OC

### 2.1.2.2 OP metabolites

In the human body, the main metabolic reaction, common to all OP, is hydrolysis of the ester bond, with the production of alkylphosphate derivatives and chemical residues specific for each compound. Most OP compounds are metabolized yielding six dialkylphosphates (DAP), as terminal products, which are excreted in urine. These six metabolites are: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) (Table 3). These DAP were among biomarkers of OP exposure used in epidemiology of children's environmental health (Wessels et al., 2003). They are suitable for biological monitoring of exposure to OP, and the determination of the alkylphosphate metabolites in urine is the most practical and widely used method to estimate the internal dose of most OP pesticides used worldwide (Angerer et al., 2007; Bouchard et al., 2006; Margariti & Tsatsakis, 2009; Dulaurent et al., 2006). However, it is necessary to understand the kinetics of metabolite excretion to know the optimum time for urine collection. Besides, not all OP are metabolized to a measurable level of DAP (Wessels et al., 2003).

In addition to DAP, some pesticide-specific metabolites of OP frequently measured in urine for biological monitoring of human population exposed to OP pesticides, such as para-nitrophenol (PNP) and trichloro-pyridinol (TCP) (Hryhorczuk et al., 2002; Aprea et al., 2002, Maroni et al., 2000a) are listed in Table 3. In general, they are approved as useful indices of recent exposure and used for risk assessment of OP pesticides as a biological indicator of exposure (Aprea et al., 2002).

### 2.1.2.3 CB metabolites

Biological monitoring of CB pesticides can be carried out by determination of their metabolites in biological samples. Specific metabolites of main carbamate compounds, measured in urine for biomonitoring of human population exposed to these pesticides are summarised in Table 4.

Metabolites	Main parent compounds
Alkylphosphates: Dimethylphosphate (DMP)  Dimethylthiophosphate (DMTP)  Dimethyldithiophosphate (DMDTP) Diethylphosphate (DEP)  Diethylthiophosphate (DETP)  Diethyldithiophosphate (DEDTP)	Malathion, dichlorvos, dimethoate, , temephos fenchlorphos, mevinphos. Azinphos-methyl, dimethoate, fenchlorphos, fenitrothion, malathion. Azinphos-methyl, dimethoate, malathion. Thion, disulfoton, parathion, phorate, terbufos quinalphos, demeton, diazinon, dichlofenthion, Diazinon, demethon, parathion, phorate, quinalphos Disulfoton, phorate
Para-nitrophenol (PNP)	Parathion Parathion-methyl
3,5,6-trichloro-pyridinol (TCP)	Chlorpyrifos Chlorpyrifos-methyl
3-methyl-4-nitrophenol (MNP)	Fenitrothion
Mono- and Di-carboxylic phosphorus acids	Malathion
Aminomethyl-phosphonic acid	Glyphosate

Table 3. Main metabolites of OP, measured in urine for human biomonitoring.

Metabolites	Parent compounds
Carbendazim, methyl (5-hydroxy-1Hbenzimidazol-2-yl) Methyl (4-hydroxy-1H-benzimidazol-2-yl)	Benomyl
1-naphthol	Carbaryl
2-dimethylamino-4-hydroxy-5,6-dimethylpyrimidine 2-methylamino-4-hydroxy-5,6-dimethylpyrimidine	Pirimicarb
2-hydroxyphenyl N-methylcarbamate 2-isopropoxyphenol	Propoxur
Carbofuranphenol	Carbofuran Benfuracarb Carbosulfan Furathiocarb

Table 4. Specific metabolites of main carbamate compounds measured in human urine.

#### 2.1.2.4 Pyrethroids metabolites

Pyrethroids are more modern insecticides which are more neurotoxic to insects and less neurotoxic to humans than organophosphates. This led to lower amounts which have to be applied and to lower exposure of the general population. The most important pyrethroids permethrin, cyfluthrin, cypermethrin and deltamethrin, are immediately hydrolysed in the human body. Several methods exist for the measurement of synthetic pyrethroid metabolites in human urine. (Leng et al., 1997; Aprea et al. 1997; Maroni et al., 2000c; Smith et al., 2002; Schettgen et al., 2002; Leng et al., 2006; Fortin et al., 2008). The metabolites 3-

phenoxybenzoic acid (3-PBA), 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA), 4-fluoro-3-phenoxybenzoic acid (F-PBA) and 3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), which are excreted in urine are commonly used as human biomonitoring parameters (Table5).

Substance	Metabolite in urine
Allethrin, bioallethrin Phenothrin Pyrethrum Resmethrin Tetramethrin	<i>trans</i> -Chrysanthemumdicarboxylic acid
Cyfluthrin Cypermethrin Permethrin	<i>cis</i> - and <i>trans</i> -3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid ( <i>cis</i> and <i>trans</i> -DCCA)
Deltamethrin	<i>cis</i> -3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid ( <i>cis</i> -DBCA)
Cypermethrin Deltamethrin Permethrin	3-Phenoxybenzoic acid (3-PBA)
Cyfluthrin	4-Fluoro-3-phenoxybenzoic acid (F-PBA)

Table 5. Pyrethroid insecticides and their corresponding metabolites

## 2.2 Biomarkers of effect

Biomarker of effect is a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease (WHO, 1993). Such markers include several specific markers for target tissues and should reflect early biochemical modifications that precede structural or functional damage. Thus, knowledge of the mechanism(s) that lead to ultimate toxicity is necessary or at least extremely important to develop specific and useful biomarkers. Many biomarkers of effect are used in everyday practice to assist in clinical diagnosis, but for preventive purposes an ideal biomarker of effect is one that measures change that is still reversible. Nevertheless, certain biomarkers of non-reversible effects may still be very useful in epidemiological studies or provide the opportunity for early clinical intervention.

A limited range of tissues is available for routine analysis of these biomarkers. The more accessible tissues are therefore used as surrogates for the known or putative target tissues. In some instances biomarkers of effect are not mechanistically related to chemically induced lesions, but may represent concomitant, independent changes (WHO, 1993).

### 2.2.1 Biological markers of OC effects

Animal studies have shown that the earliest biological modification after exposure to OC pesticides is a dose-dependent induction of the activity of certain microsomal enzymes. This effect can be measured by indirect methods, such as the determination of 6- $\beta$ -hydroxycortisol and D-glucaric acid excretion in urine, or the measurement of blood half-life of the test drugs. Enzyme induction has been documented in workers with repeated exposure to aldrin/dieldrin, endrin, lindane and DDT. However, these tests are not specific

for OC exposures because several other xenobiotics (e.g. alcohol) or drugs (e.g. barbiturates) have inductive effects on the liver microsomal enzymes. The only specific test for the biological monitoring of human exposure to OC pesticides is the measurement of the intact compounds or their metabolites in biological samples. Since hexachlorobenzene can cause chemical porphyria in man, the change in urinary excretion of porphyrins has been proposed as an early biomarker of effect (Maroni et al., 2000d).

### 2.2.2 Inhibition of cholinesterase activity

Measurements of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities have been used as primary biomarkers to take necessary measures in the field of occupational as well as emergency medicine in cases of clinical poisoning and accidental OP and/or CB exposure (Hernandez et al., 2005; Souza et al., 2005; Safi et al., 2005; Stefanidou et al., 2009; Ng et al., 2009; Simoniello et al., 2010; Ueyama et al., 2010). Blood cholinesterases have been widely used for monitoring exposure to OP and CB pesticides. Strong associations were reported between exposure to these compounds and symptoms of chronic pesticide toxicity, and cholinesterase activities were significantly reduced in exposed populations (Hernandez et al., 2004, 2005; Remor et al., 2009).

The AChE is enzyme instantly performs the hydrolytic cleavage of acetylcholine, the chemical mediator responsible for the physiological transmission of nerve action potential. The main mechanism of action of OP and CB compounds is inhibition of cholinesterase activity. Different OP pesticides may inhibit AChE or BChE to a different degree (Costa et al., 2005). Nearly all OP insecticides cause toxic effects in humans through the inhibition of AChE in the nervous system. When OP insecticides are present, AChE is phosphorylated and is no longer able to break down acetylcholine into choline and acetic acid. The resulting accumulation of endogenous acetylcholine is responsible for the typical signs and symptoms (muscarine-like effects and nicotine-like effects) occurring after acute poisoning (cholinergic over-stimulation syndrome) (Maroni et al., 2000d).

BChE, also known as pseudo-cholinesterase, is found in plasma. The physiological function of this enzyme in blood is yet to be discovered (Costa et al., 2005). Depression of the BChE enzyme activity is not necessarily associated with symptoms of anti-cholinergic toxicity, and large depressions in BChE have been noted in the absence of any effect on erythrocyte AChE (Soltaninejad et al., 2007). The BChE is still a useful biomarker to predict and prevent health hazards of workers chronically and occupationally exposed to pesticides (He, 1999; Ranjbar et al., 2002; Rastogi et al., 2008; Araoud et al., 2011). The plasma cholinesterase might be a more sensitive indicator of exposure to some OP compounds such as malathion, diazinon and dichlorvos (He, 1999; Costa et al., 2005; Hernandez et al., 2006; Jintana et al., 2009). According Jintana et al. (2009), the effect of OP exposure on cholinesterase activity was found predominantly in BChE. One of the possible reasons is that potential inhibition of AChE and BChE varies widely among the different OP compounds. Moreover, some OP inhibits BChE more strongly than AChE. The inhibition of BChE is highly correlated with intensity and duration of higher exposure to a large group of OP and carbamate pesticides. Ancientness of exposure as well as type of pesticides used were also implicated in a significant variation of BChE activity and can be considered as risk factors of exposure to pesticides (Araoud et al., 2011). However, BChE inhibition does not mirror the biological effects of OP in the nervous system (He, 1999). On the other hand, AChE is better than BChE for the assessment of chronic exposure to OPs, since a cumulative inhibition is observed due to its lower recovery rate compared to that of BChE (Kamel & Hoppin, 2004).

### 2.2.3 Inhibition of neuropathy target esterase activity

In addition to cholinergic effects, certain OP can cause another type of neurotoxicity, a central peripheral distal sensory-motor axonopathy, known as organophosphate-induced-delayed-polyneuropathy (OPIDP). OPIDP is not related to inhibition to AChE but rather is associated with phosphorylation of another esterase denominated neuropathy target esterase (NTE) in the nervous system (Costa et al., 2005). In humans, NTE is present in the nervous tissue, liver, lymphocytes, platelets and other tissues. Its physiological function, if any, is still unknown (Maroni et al., 2000a). Moreover, limited information is available on the degree of inter- and intra-individual variation (Costa et al., 2005).

The initial biochemical reaction is represented by the phosphorylation of NTE, while the second step is the transformation of the phosphorylated target into an 'aged' form. The ageing reaction depends on the chemistry of OP pesticides and may only occur with phosphate, phosphonates and phosphoramidates. Compounds such as, sulphonates and carbamates are not able to age and, if they are linked to NTE before an axonopathic OP compound is administered, they block the receptor preventing the development of the neuropathy (Maroni et al., 2000a). For the OP compounds causing delayed polyneuropathy, measurement of the activity of NTE in lymphocytes appears to offer a useful marker for biological monitoring and for predicting purposes (Costa & Manzo, 1995).

### 2.2.4 $\beta$ -Glucuronidase

$\beta$ -Glucuronidase (BG) was isolated from microsomal, Golgi, lysosome, and eventually released into the serum fraction (Ueyama et al., 2010). It has been previously reported that BG activity could be considered as a novel biomarker of anticholinesterase pesticides exposure. This enzyme is stabilized within the luminal site of the microsomal membrane by a complex with egasyn, one of the carboxylesterase isoenzymes (Hernandez et al., 2004; Ueyama et al., 2010). Exposure to OP or CB insecticides is followed by the cleavage of the egasyn-glucuronidase complex, leading to a rapid increase in plasma BG activity (Hernandez et al., 2004). The mechanism involved was not fully understood at that time. A massive increase in plasma BG was noted in rats within a few hours after paraoxon or parathion-treatment (Ueyama et al., 2010). In human, a significant increase in blood BG activity was reported in acute OP poisoning cases (Soltaninejad et al., 2007). These authors suggested that BG is very sensitive biomarker at high exposure to OP. Recently, Ueyama et al. (2010) explained that plasma BG activity, in a group of chronically OP-exposed farmers, was significantly increased compared to that in controls. Hence, they indicated that the measurement of BG activity is a practical approach to detect low-level occupational exposure, at which no BChE inhibition is observed. Blood BG can be a more sensitive biomarker of OP exposure than the inhibition of AChE and BChE activities in rats as well as humans (Ueyama et al., 2010).

### 2.2.5 Cytogenetic biomarkers

Cytogenetic markers such as chromosomal aberrations (CA), sister chromatid exchange (SCE), micronuclei (MN) and single cell gel electrophoresis SCGE or Comet assay have been extensively used for the detection of early biological effects of DNA-damaging agents. Regarding pesticide exposure, a positive association between occupational exposure to complex pesticide mixtures and the presence of CA, SCE and MN, has been detected in the majority of reports, providing suggestive evidence of genotoxic effects induced by pesticides (Bolognesi, 2003; Pastor et al., 2003; Das et al., 2007; Remor et al., 2009).

The Comet assay has been used to determine the extent of DNA damage in lymphocytes from farmers with occupational exposure to a variety of pesticides (Angerer et al., 2007). Occupational exposure to pesticides resulted in a significant increase in DNA and chromosome damages in blood cells (Paz-y-Mino et al., 2004). Studies of environmental pesticide exposure, using Comet assays of leukocytes, showed a positive correlation between DNA damage and dichlorodiphenyl dichloroethylene (DDE), dichlorodiphenyl dichloroethane (DDD), and dichlorodiphenyl trichloroethane (DDT) levels. However, exposure to deltamethrin did not induce DNA damage (Valverde & Rojas, 2009). But, according Villarini et al. (1998), the *in vitro* genotoxicity of deltamethrin evaluated by assessing the ability of the insecticide to damage DNA, using the SCGE or SCE assay and MN test in human peripheral blood leukocytes, revealed that this pyrethroid insecticide has a weak genotoxic activity and is not completely devoid of long-term effects as a consequence of its interaction with DNA (Villarini et al., 1998). Generally, since workers are frequently exposed to complex mixtures of pesticides, it is difficult to attribute the genotoxic damage to any particular chemical class or compound.

### 2.2.6 $\delta$ -aminolevulinic acid dehydratase

Changes in erythrocyte  $\delta$ -aminolevulinic acid dehydratase (ALA-D), an erythrocyte enzyme, have been reported after exposure to different pesticides both *in vitro* and *in vivo* (Hernandez et al., 2005; Remor et al., 2009). The inhibition of ALA-D (40%) shortly after the administration of paraquat has been attributed to the generation of oxidative stress (Noriega et al., 2002, as cited in Hernandez et al., 2005). The results of the study of Hernandez et al. (2005), indicate that exposure to pesticides in an intensive farming setting could lead to a reduction in erythrocyte ALA-D. The decrease of ALA-D observed may partly be due to non-competitive binding of pesticides to the enzyme, since these chemicals are neither substrates nor competitors of substrates. However, the vicinal sulfhydryl of ALA-D may have been modified by the exposure leading to inhibition of enzyme activity (Hernandez et al., 2005). The relevance of this inhibition is unknown, although a failure of heme synthesis could account for the haemogram changes previously reported in the context of long-term exposure to pesticides (Parron et al., 1996). Therefore, the ALA-D may become an important sensitive biomarker that can be used together with AChE and/or BChE for the assessment of long-term health risks of workers exposed to pesticides.

### 2.2.7 Other biomarkers

Several additional biochemical and haematological parameters present in human biological fluids were used as biomarkers to detect early effects of pesticides before adverse clinical effects occur. Parron et al. (1996), reported decrease in the mean corpuscular haemoglobin concentration (MCHC) and in the mean platelet volume (MPV) in respectively, 38% and 15% of greenhouse sprayers chronically exposed to pesticides. The main alterations found in the total and differential white blood cell count were the increase of monocytes in 5% of workers and of eosinophiles in 4% of these sprayers (Parron et al., 1996).

Studies on human toxicity of pesticides had also focused on biological parameters related to organ functions. The biochemical dysfunctions could reflect either hepatic or renal cytotoxicity. A subtle nephrotoxic changes in workers occupationally exposed to pesticides was reported, because of their higher levels of serum creatinine and/or blood urea (Hernandez et al., 2006). Altered liver enzyme activities, such as serum alanine

aminotransferase (ALT) and aspartate aminotransferase (AST), have been reported among pesticide workers exposed to OP alone or in combination with OC or other pesticides (Anwar, 1997; Altuntas et al., 2003; Khan et al., 2008). The presence of the pesticide in the body affects the metabolism of tryptophan leading to hyperglycemia and affect the ALT, AST, gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) activities (Tsatsakis et al., 2009). An increase in triglycerides levels, GGT activity, and inorganic phosphorus levels was reported in 17%, 8% and 7% respectively, of a cohort of pesticide sprayers (Parron et al., 1996). Friedman et al. (2003) reported elevations in creatine kinase (CK) in patients more than 10 years after acute exposure to anticholinesterases.

### 2.3 Biomarkers of susceptibility

Biomarkers of susceptibility constitute an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance (WHO, 1993). They are of special interest as they are genetically determined and may predispose to an increased risk in the case of exposure to pesticides (Hernandez et al., 2003). These biological markers indicate which factors may increase or decrease an individual's risk of developing a toxic response following exposure to pesticides. The use of these biomarkers, reflecting genetically linked or acquired susceptibility to pesticides or their metabolites, provides an opportunity for the recognition and protection of sensitive individuals (WHO, 1993).

Pesticides undergo bioactivation and detoxification processes that can be affected by genetic polymorphisms in biotransformation enzymes. Genetic variations in such enzymes as well as in the enzymes that are targeted by pesticides can greatly influence their toxicity and would, therefore, render an individual more or less susceptible to adverse effects of these compounds (Costa et al., 2005). Pesticide metabolism studies conducted with human provide valuable information as to metabolic pathways and the enzymes involved in these pathways, and can aid in the identification of individuals that may have increased risk after exposure to pesticides (Rose et al., 2005).

Individual susceptibility has been reported to play a critical role in the assessment of exposure to pesticides, because of at the same exposure level individual susceptibility determines whether or not clinical symptoms or even intoxication appear. This individual susceptibility is caused by polymorphic key enzymes like esterases and transferases as well as their synergisms. Most studies have focused on the influence of isolated enzyme activities on the toxicokinetics of the foreign substance; however, there is little data on the combined effects of a number of polymorphic enzymes mainly involved in the detoxification of pesticides (Lewalter & Leng, 1999; Hernandez et al., 2005). Inheritance of the unfavourable versions of the different polymorphic genes has been associated with an increased activation or reduced detoxification and elimination of pesticides, and could entail an increased susceptibility to pesticides (Bolognesi, 2003). Although all the pathways for pesticide detoxification are not fully understood, the process involves three main systems: the cytochrome P450 enzymes, glutathione S-transferases, and the esterases system that metabolizes insecticides.

#### 2.3.1 Cytochrome P450 enzymes

Many pesticides are converted to active intermediates via reactions catalyzed by cytochrome P450 (CYP450). Variations in P450 enzymes have considerable *in vivo* effects on the

sensitivity of humans to chemical toxicity. Therefore, some humans may be at increased risk of toxicity due to their P450 enzymes profiles (Mutch & Williams, 2006). CYP450 enzymes metabolize some carbamate and nicotinoid insecticides (Hodgson, 2003). However, these enzymes catalyse mainly the oxidative desulfuration of organothiophosphates to the corresponding oxons (Figure 2) and also mediate detoxification of several OP. Both bioactivation and detoxification of OP are mediated by multiple CYP450, some of which present polymorphisms that can confer differences in catalytic activity or level of expression of various substrates (Costa et al., 2005). CYP1A2 and members of CYP2 and CYP3 families are involved in activation as well as detoxification some of OP such as parathion, which is converted to paraoxon by the combination of CYP2C8, CYP3A4/5, CYP2D6 and CYP1A2 (Nebert, 2005). The functional expression of CYP450 in human liver will influence formation of the oxon and its systemic concentration. Therefore, the individuals' profile of participatory P450 will more accurately predict susceptibility to OP toxicity, and such information will be useful to complement risk assessment of OP exposures (Mutch & Williams, 2006). A number of pesticides may modify the cancer risk through the altered CYP2E1 enzyme activity (Bolognesi, 2003). However, the significant increase in DNA damage found in population of pesticide sprayers was not significantly influenced by genetic polymorphisms of the CYP1A1 gene (Paz-y-Mino et al., 2004).

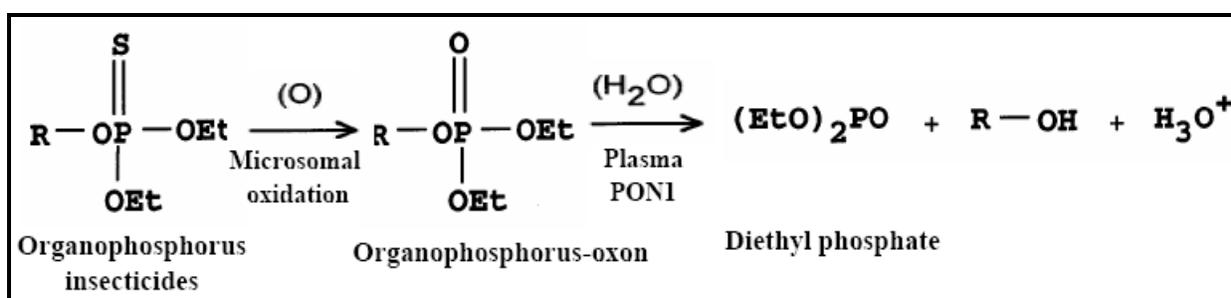


Fig. 2. The cytochrome P450/PON1 pathway for the bioactivation and subsequent detoxification of several organophosphorus insecticides.

## 2.3.2 Esterase

### 2.3.2.1 Cholinesterase

Cholinesterase variants are known to be responsible for the individual susceptibility regarding the occurrence and severity of the related symptom. A large number of polymorphisms have been described for BChE target for OP. In addition to the wild-type allele, there are at least 39 identified genetic variants with nucleotide alterations in the coding regions (Lockridge & Masson, 2000). Most of these variants, which are usually grouped in four categories (silent, K-variant, atypical and fluoride), are silent, i.e. they have 0 or less than 10 % of normal activity. Limited evidence in animals and humans suggests that individuals with genetic variants of BChE that have reduced activity may be more sensitive to OP toxicity (Lockridge & Masson, 2000). A genetic variant of human AChE has been described, which is not associated with alterations in enzyme activity (Bartels et al., 1993, as cited in Hernandez et al., 2005). However, based on work in AChE knockout mice, it has been suggested that silent AChE alleles may exist in humans, and may be associated with increased sensitivity to OP toxicity (Costa et al., 2005).

### 2.3.2.2 Carboxylesterases

The carboxylesterases (CbE), are members of the serine hydrolase superfamily of esterases that metabolize ester-containing xenobiotics such as pyrethroids, and they are implicated in the detoxification of a number of OP insecticides. These hepatic enzymes are expressed abundantly in the mammalian liver and utilized by insects to gain resistance against insecticides (Ross et al., 2006; Zhou et al, 2007).

Individual susceptibility plays a major role in pyrethroid induced adverse effects like skin paresthesia. For pyrethroids, marker of susceptibility is not well known yet. The determination of the carboxylesterase activity in human isolated lymphocytes was a first step in the search of a marker of pyrethroid susceptibility in man (Leng et al., 1999). The study of Ross et al., (2006) has demonstrated that hCbE-1 and hCbE-2 are human pyrethroid-hydrolyzing CbE and that these enzymes will be useful biomarkers of susceptibility in populations that are occupationally and environmentally exposed to these xenobiotics.

### 2.3.2.3 Paraoxonase

Paraoxonase1 (PON1) is a member, of family of proteins that includes PON2 and PON3. This enzyme, which takes its name from its most studied substrate, paraoxon, is capable of hydrolyzing the oxygen analogs of a number of commonly used OP (Figure2) as well aromatic esters and carbamate insecticides (Hernandez et al., 2004; Costa et al., 2005). Variations in PON1 activity may contribute to interindividual variations in susceptibility, and this detoxifying enzyme was considered as one important biomarker of susceptibility to long term exposure to pesticides (Leng et al., 1999; Hernandez et al., 2003; Costa et al., 2005; Sirivarasai et al., 2007; Zhou et al, 2007; Perez-Herrera et al., 2008). This enzyme exhibits substrate-dependent genetic polymorphisms, both in coding and promoter regions, leading to changes in PON1 activity and level. The Q192R substitution has been shown to be responsible for the substrate-dependent activity polymorphism. The PON1 192R isoform hydrolyzes paraoxon more rapidly than the PON1 192Q isoform, whereas the PON1 192Q isoform hydrolyzes diazoxon, soman and sarin more rapidly than the PON1 192R (Costa et al., 2005; Sirivarasai et al., 2007). The polymorphism at position 55 (L55M) does not affect catalytic activity, but has been associated with plasma PON1 protein levels, with PON1 55M being associated with low plasma PON1. The polymorphism at position -108 (T/C), in the promoter region of PON1, is the major contributor to differences in the level of PON1 expression, and appears to have the major effect on the levels of PON1 found in plasma of individuals.

Although PON1 activity remains stable over time within a given individual, it varies 10 to 40 fold between individuals. PON1 genotype is an important determinant of a farmworker's susceptibility to chronic pesticide poisoning, as PON1Q allele is an independent predictor of chronic toxicity (odds ratio 2.9; 95% confidence interval: 1.7-6.7) (Lee et al., 2003). Since the genetic polymorphisms of PON1, at position 192 and -108, infer different catalytic activity and levels expression, it is reasonable to assume that some individuals in the population will exhibit a significantly increased sensitivity to OP exposure (Costa et al., 2005). It has been also reported that the health status of individuals after exposure to OP pesticides is related to the polymorphism of PON1. Lee et al. (2003) have examined the association between PON1 genotype and symptoms of chronic pesticide toxicity in pesticide-exposed workers. A significantly higher proportion of subjects with the slow genotype reported two or more symptoms of chronic toxicity compared to those with fast PON1 activity. Moreover,

the genetic variants of this pesticide metabolizing enzyme may confer a predisposition factor to Parkinson disease, and thus, is considered candidate gene for association studies. An interaction, between OP exposure and PON1Q192R polymorphism, was found on adverse effects on sperm DNA integrity in agricultural workers, and these individuals featuring the 192RR genotype were more susceptible to develop reproductive toxic effects by OP exposure (Perez-Herrera et al., 2008). According Chia et al. (2009), PON1 polymorphisms differed among ethnic groups, implying that ethnicity could be an important surrogate for identifying susceptible groups in case of OP exposure.

All these reports pinpoint the potential usefulness of PON1 genotyping as a biological indicator of susceptibility to long term exposure to OP. Thus, genotyping individuals for PON1 polymorphisms may provide a method for the identification of individuals with the high risk of OP poisoning.

### 2.3.3 Glutathione S-transferases

This family of enzymes presents genetic polymorphisms in human populations responsible for the glutathione conjugation of various reactive species of many chemicals including pesticides (Bolognesi, 2003; Schroeder, 2005). Several glutathione S-transferases (GST) polymorphisms have been identified. The GSTP1 polymorphisms, affecting substrate selectivity and stability, may increase the conversion of a pesticide to a toxic metabolite and hence, can increase the toxicity of some substrates. These enzymes have been investigated as risk factors for and non-Hodgkin's lymphoma in individuals exposed to pesticides (Schroeder, 2005). The significant differences in GSTP1 genotype frequencies shown in patients with Parkinson's disease exposed to pesticides, might explain susceptibility to Parkinson's disease after pesticide exposure (Menegon et al., 1998). The polymorphisms of GST mu and theta (GSTM1 and GSTT1) were studied as biomarkers of individual susceptibility in professional applicator of pesticides. Null genotype for both GST subclasses (GSTM1 and GSTT1) was found to be the unique independent predictor of pesticide-related symptomatology. Moreover, GSTT1 was a relevant determinant of susceptibility to chronic pesticide poisoning (Hernandez et al., 2005). Liu et al. (2006) revealed that metabolic GSTP1 gene may modulate DNA damage in pesticide-exposed fruits growers. The GST may play a role in the detoxification of certain OP, particularly those with a methyl group in the alkyl chain. Indeed, the potential significance of human GST polymorphisms as determinants of individual difference in human susceptibility of OP has not been well investigated (Costa et al., 2005).

### 2.3.4 Gene-gene interactions

Enzymes involved in pesticide metabolism have overlapping substrate specificities, and the lack of one-to-one specificity may buffer effects of any single metabolism enzyme variant by providing an alternate catalyst for metabolic transformation. Likewise, effects of "high-risk" polymorphisms affecting a single metabolic step may be diminished by "low-risk" variants acting at subsequent steps, such that consequences of rapid phase I activation may be lessened by rapid phase II detoxification. Allelic variants of CYP1A1 and PON1 have been studied as susceptibility factors in pesticides toxic responses. Tsatsakis et al. (2009) has reported an association between the CYP1A1/PON1 polymorphisms and various clinico-pathological findings, in rural population professionally exposed to pesticides. According to Schroeder (2005), PON1 and BChE enzyme variants, associated with altered activity and

acute OP toxicity, are strong candidate susceptibility factors for pesticides and non-Hodgkin's lymphoma (Schroeder, 2005). Therefore, it may be necessary to evaluate joint effects of multiple functional gene polymorphisms to detect an effect on pesticide metabolism and impaired health.

### 3. Conclusion

The biological monitoring is becoming an increasingly important element of field studies designed to assess the risk from pesticide exposure for preventive purposes. The use of biomarkers as an integrated measure of exposure and/or effects is increasing as a result of difficulties in exposure source identification and demands of more integrated data for risk assessment. This is inevitable in environmental health, with often unknown and very mixed exposures, but also occupational health implies mixed exposures and several routes of exposure, impossible to detect by conventional environmental monitoring. Biomonitoring data will be valuable in making associations with health effects and risk of adverse outcome. Therefore, the biological markers must provide the critical link between chemical exposure, internal dose and health impairment, and are of value in assessment of risk. However, there is a need to identify and validate for each organ system those characteristic parameter(s) that are indicative of induced dysfunction, clinical toxicity or pathological change, as well as to establish the specificity and sensitivity of each biomarker and its method of measurement. Thus, clinical issues connected with acute poisoning, identification of long-term effects, the development and validation of new and effective biomarkers of human exposure, effect and susceptibility, and study of interaction mechanisms are areas in which continuing research is warranted in order to improve the quantitative risk assessment to protect human health.

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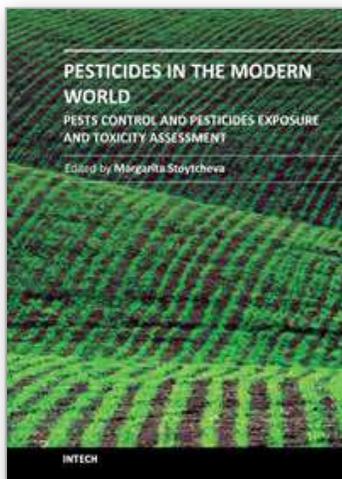
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**Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment**

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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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