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Biomarkers of Pesticide - Contaminated Environment

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1. Introduction

Pesticide is the umbrella term for chemicals or biologicals used to control pests. The Environmental Protection Agency (EPA) defines a pesticide as any substance or mixture of substances/chemicals intended to prevent, destroy, repel or mitigate any pest (US-EPA, 2006). A pesticide need not always kill a pest: it could sterilize, or repel. Pesticides can be classified in various ways such as, by their target, chemical nature, physical state and mode of action (Ware, 2000). Classification based on the target is perhaps the most widely known as the following examples indicate; Pesticides used to manage insects are called insecticides; and those used to manage rodents are called rodenticide; those used to manage fungi are called fungicides e.t.c (Ware and Whitacre, 2004). Pesticides also include plant growth regulators, defoliants, or desiccants (Hagtrum and Subramanyam, 2006).

The environmental pollution and poisoning owing to the widespread use of pesticides in agricultural and domestic pest control may be detrimental to the health of handlers, non-target organisms and consumers. Pesticides or their residues are ubiquitous contaminants in the environmental media (air, soil, water), and in humans, plants and animal tissue samples. Pesticides uptake occurs through the skin, eyes, by inhalation, or by ingestion directly or through the food chain. The fat-soluble pesticides, and to some extent, the water-soluble pesticides are absorbed through intact skin. Sores and abrasions may facilitate uptake through the skin. The vapours of pesticides or aerosol droplets smaller than 5µm in diameters are absorbed effectively through the lungs. Larger inhaled particles or droplets may be swallowed after being cleared from the airways. A common toxic effect to the lung is the result of oxidative burden which occurs as a result of active oxidants in pesticide mixtures, especially free radicals that are generated by a variety of toxic agents and the action of lung defense cells. Much of the oxidative damage to lungs is probably done by free radicals, such as hydroxyl radical, HO·; and superoxide ion, O₂⁻ which initiate and mediate oxidative chain reactions. Lungs of animals exposed to oxidants have shown elevated levels of enzymes that scavenge free radicals, providing evidence for their role in oxidative damage. There is evidence to suggest that lung cells damaged by toxicants release species that convert lung O₂ to reactive superoxide anion. Pesticide ingestion can occur from the consumption of contaminated food or from using contaminated utensils. Contaminated

hands may also lead to an intake of pesticides, for example, while palm-chewing, tobacco eating or smoking, spraying, mixing, or handling the pesticides. A number of pesticides have been identified as probable and possible carcinogens, disruptors of endocrine and immune functions, genotoxins and developmental and reproductive toxins. The improper use of pesticides may engender biological effects beyond those for which they were originally manufactured. Humans and other non-target organisms are diverse in their responses to exogenous exposures because of variability in the rate of metabolism (Otitoju and Onwurah 2007).

The use of man-made chemicals for obvious positive reason(s) has inadvertently resulted in creating negative effects in the environment, and hence the need for development of methods to assess, monitor and mitigate the impact. Pesticide exposure in humans can be measured in two ways, either through direct monitoring by measuring biomarkers from individuals or developing models (animals, plants or microorganisms) to assess exposure. Biomarkers, or biological markers, are biochemicals or metabolites that can be measured in body fluid, such as urine, blood, saliva, and other body fluids. Indicator organisms also serve as biomarkers. The definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to any xenobiotic exposures. Biochemical biomarkers are increasingly used in ecological risk assessments of aquatic and terrestrial ecosystems to identify the incidence of exposure to and effects caused by xenobiotics. Until recently, the most common end point measured when evaluating toxicity of chemicals were mortality values. This, unfortunately can only provide a measure of short-term acute toxicity and are not always useful for predicting the ecological consequences of exposure to a particular chemical, e.g., as seen with reproduction, where effects are observed at concentrations well below the lethal concentration (LC₅₀) value. Biomarkers can provide information on the potential adverse impacts of contaminants and can act as early warning signals of impending environmental damage. Pesticides inhibit a number of enzymes in humans. They affect several physiological systems and processes in the body – the central nervous system (CNS), reproductive, immune, endocrine, cardiovascular and respiratory systems.

The repertoire to counteract the potentially hazardous reactions initiated by metabolites from pesticide oxidation include all levels of protection, prevention, interception and repairs by certain antioxidant enzymes or biomarkers, such as glutathione S-transferase (GST), and superoxide dismutase (SOD). Glutathione S-transferase is a family of detoxifying enzymes that catalyzes the conjugation of reduced glutathione with a group of compounds having electrophilic centers e.g., nitrocompounds, organophosphates and organochlorides. Since glutathione (GSH) is essential to cellular detoxification of many toxic xenobiotics, monitoring this endogenous thiol during pesticide exposure is very important. Hence, the aim of this chapter is to evaluate some antioxidant enzymes and vitamins reported as biochemical markers for pesticide toxicity.

2. Need for biomarker development

Pesticide poisoning issue in developed and developing countries has received several attentions as can be seen from some of many press statements. Several people in crop-growing areas where pesticides are used indiscriminately almost have constant nausea, diarrhea and dizziness because persistent exposure to pesticides has depleted their immune system. There

have been several reports about pesticides making people in agricultural areas extremely ill, and experts have "no doubt" that exposure to many of the pesticides commonly used damage human health. Some researchers at the University of Cape Town's Occupational and Environmental Health Research Unit, reported that exposure to pesticides is bad for human health. Farmers might put up barriers such as trees, but the poisons could accumulate in the water or dust, and children crawling on the ground would be exposed to them.

The World Health Organisation receives reports of about three million cases a year of acute pesticide poisoning, most from developing countries, and estimates that about 20,000 people die every year from pesticide exposure. Until the 1970s, farmers in South Africa targeted pests by using equipment carried on a backpack to spray plants individually with pesticides. These days, it is normal to spray from tractors, a method that produces clouds of pesticide that can travel for kilometres. In Groblersdal, Limpopo, where citrus, grapes, cotton, vegetables and maize are grown, teenagers and a five-year-old who were growing breasts were diagnosed of chronic fatigue, nausea, muscle aches and pains, skin rashes and arthritis, particularly during the spraying season. Regular testing of their blood revealed that they have been exposed to organophosphates which attack the nervous system, and carbamate pesticides. One commonly used pesticide, endosulphan, has been shown to be linked to reproductive impairments.

The above calls for caution in the use of pesticides and abide to safety precautions without endangering the lives of people. The health and safety of the farm workers and neighbours should always have priority over the profit motive of the farmers. This is the reason scientists should develop more accurate and robust biomarkers for pesticides' toxicity assay and management so that a Policy that will pave a way for a Bill on pesticides could be passed.

3. Biomarkers

One of the greatest challenges to humanity today is the endangerment of human health due to indiscriminate use of pesticides. To estimate the biological danger thereof, knowledge of their harmful effects is necessary. In revealing the risks of such substances, every living being and life function can be considered a potential biomarker or bioindicator. Microorganisms can be used as indicator organisms (or biosensors) for toxicity tests or in risk assessment. Bioassays are ideal complement to the traditional analytical techniques employed in evaluating toxicity of pollutants or chemicals in the environment. Those tests performed with bacteria are considered to be the most reproducible, sensitive, simple, economical and rapid (Matthews, 1980). Risk assessment has relied on models that use toxicity data and physical properties of chemicals, and this approach has been effective at the ecosystem level. The use of microorganisms present in a polluted environment is an approach that provides a link between exposure and effect because chemicals are known to elicit measurable and characteristic biological responses in exposed (microbial) cells. The term "biological markers" (or biomarkers) can be taken to mean cellular, biochemical or molecular alterations) which are measurable in biological media such as the human tissue, cells or fluids as a result of exposure to environmental chemicals (Hulka, 1988). Three types of events involved are exposure, effect, and susceptibility (Schultz and Mazzuckelli, 1991). In a broad sense, biological markers are measurements in any biological specimens (such as the blood plasma, bacterial cells) that will elucidate the relationship between exposure and effect such that adverse effects could be prevented (NRC, 1992).

A crucial aspect of Ecotoxicology is the measurement of the effects of toxic substances on organisms in ecosystems and on ecosystems as a whole. This has traditionally been done by determining levels (or bioaccumulation) of toxic substances in organisms and relating these levels to detrimental effects on the organisms (biomarkers). Biomarkers can be used to identify causal associations and to make better quantitative estimates of those associations at relevant levels of exposure. They may also make it possible to identify susceptible groups or individuals who are at risk of exposure to certain types of environmental and occupational agents (Anwar, 1997). A better approach is the use of biomarkers consisting of observations and measurements of alterations in biological components, structures, processes, or behaviours attributable to exposure to xenobiotic substances as shown in Figure 1. Animals, microorganisms or plants can be used as biomarkers to evaluate the effect of chemical hazards to humans. Biomarkers, or biological markers, can also be chemicals or metabolites that can be measured in body fluid, such as urine, blood, saliva, and other body fluids. Metabolites are chemicals that were transformed by the body from an original chemical, or chemical constituents of the pesticide. The biological events detected can represent variation in the number, structure, or function of cellular or biochemical components. Recent advances in molecular and cellular biology allow for measurement of biologic events or substances that may provide markers of exposure, effect, or susceptibility in humans. Certain tests, such as DNA adduct formation, are used for measuring biologically effective dose, whereas others are considered to measure early effects, such as chromosomal aberrations. Biomarkers are predictive assays rather than diagnostic. A positive effect will be

Fig. 1. Pathway of Biomarker responses after toxic interaction

indicative of exposure, but cannot be considered predictive of the future occurrence of any particular change in phenotype such as cancer. Biomarkers are used for a variety of reasons. However when assessing the toxicity of any pesticide, three types of biomarkers, each measuring different types of effects, can be distinguished and they include biomarkers of exposure, response, and susceptibility.

4. Biomarkers of exposure

A biomarker of exposure consists of the measurement of a xenobiotic substance, a metabolite of a xenobiotic or pesticide substance, or an effect directly attributable to such a substance in an organism. For example, a biomarker of human exposure to aniline might consist of measurement of it or its *p*-aminophenol metabolite in blood or urine. Pesticides or their metabolites can be measured directly in tissues obtained by biopsy, in live organisms or necropsy of deceased organisms. Urine, blood, exhaled air, faeces, and breast milk can also serve as samples and are advantageous in measurements to be made at intervals over a period of time. A particularly useful kind of biomarker used with increasing frequency during recent years consists of adducts of xenobiotics or their metabolites to biomolecules. A particularly direct example of such adducts measured for many years as evidence of exposure is carboxy-haemoglobin (COHb), produced when inhaled carbon monoxide adds to blood haemoglobin (Hb):

The COHb has a distinctly different colour from the oxygenated form, O₂Hb, and can be measured spectrophotometrically. Cancer-causing compounds and carcinogenic metabolites are generally electrophilic (electron-seeking) species that cause the biochemical changes leading to cancer by adding to nucleophilic groups (electron-rich bound oxygen and nitrogen atoms) on biomolecules, particularly those in DNA. These adducts and adducts to haemoglobin can be measured as biomarkers of exposure.

In toxicity testing, doses of pesticides are assumed to be the actual level of exposure. This is fairly accurate, but it should not be presumed that 100% of the chemical has been absorbed by the subject. For this reason blood tests are conducted to achieve a more exact estimate. This level is usually similar to amounts dispersed to organs. The absorption rate of pesticides depends upon the route of exposure. However, absorption varies more through the dermal route. When alachlor was applied to the skin of rats at a low dose (14 mg/kg) nearly 75% was absorbed, while monkeys only absorbed 8 to 10% through the skin.

5. Biomarkers of response or effect

Biomarkers of effect are alterations of physiology, biochemistry, or behaviour directly attributable to exposure to a xenobiotic substance. For example, exposure to aniline can be determined by measuring it or its *p*-aminophenol in blood, but it can also be measured by its production of blood methaemoglobin (a product of haemoglobin) useless for carrying oxygen in blood in which the Fe²⁺ in haemoglobin has been oxidized to Fe³⁺. Exposure to substances such as nerve gas organophosphates, organophosphate insecticides, and carbamate insecticides that inhibit cholinesterase enzymes required for nerve function can be determined by measurement of cholinesterase enzyme activity as a biomarker. Exposure to the insecticidal DDT metabolite, *p,p'*-DDE can be measured in the laboratory rat (*Rattus*

rattus) by induction of enzymatic cytochrome P-450 2B, an enzyme used by some organisms to detoxify xenobiotics.

Biomarkers of response are consequences of the exposure. An area of great concern for alachlor is its effects on genes which may be the basis for carcinogenesis. Alachlor is thought to be a probable carcinogen. The development of stomach, thyroid, and nasal tumors has occurred in rats given high doses of alachlor. Carcinogenesis causes an uncontrolled proliferation of cells in tissues and organs. Many cancers are caused from mutated somatic cells which disturb the genetic control. However, studies have shown that alachlor does not appear to be mutagenic. Reactions to alachlor are invoked by threshold sensitive mechanisms. Carcinogenic reactions only occur at high levels of exposure. Pharmacokinetic studies have shown that alachlor and its metabolites are transformed into a diethyl quinoneimine (DEIQ) metabolite. The presence of DEIQ produces protein adducts that lead to cytotoxicity, cell proliferation and tumor formation.

6. Biomarkers of susceptibility

Closely related to biomarkers of effect are biomarkers of susceptibility, which indicate increased vulnerability of organisms to disease, physical attack (such as low temperatures), or chemical attack from other toxicants. The most obvious biomarkers of susceptibility are those associated with weakened immune systems, which may make organisms more vulnerable to cancer, infectious diseases, or parasites. They can be seen in variations across species. Studies across different species have shown why alachlor may be more likely to be a carcinogen in certain animal species as against humans. For instance, rats are able to convert the secondary sulfide metabolite of alachlor to 2,6 - DEA, the precursor to DEIQ greater than 30 times that of monkeys and 751 times greater than humans. Variations in individual, human susceptibility may also be a result of differences in metabolism, expression of tumor suppressor genes (pharmacogenetics) and nutritional variations. Differences in metabolic phenotype, as detected in enzymes, may cause variances in required metabolic activation. Environmental carcinogen susceptibility may also have to do with phenotypes for detoxifying enzymes.

Biochemical responses to environmental chemicals (biochemical biomarkers) provide a measure of toxic effect. They are particularly valuable when used to measure the toxic effects of chemicals in the field, employing nondestructive sampling methods. A widely used biochemical biomarker is cholinesterase depression, which may involve destructive sampling (brain acetylcholinesterase [AChE]) or nondestructive sampling (serum butyrylcholinesterase). From enzyme inhibition data, it is apparent that field workers and pesticide users were exposed to significant levels of synthetic and natural toxicants. Therefore, the relationship between red blood cells (RBC), SOD, GST, catalase, vitamins A, C, E, and Zn depletion suggests a compromise of the antioxidant enzyme status.

7. Antioxidants as surrogate biomarkers

During the break down of xenobiotics and under normal metabolic processes, highly reactive compounds called free radicals are produced in the body. These compounds are inherently unstable since they have an odd number of electrons, but in order to make up for their shortage in electrons, free radicals will react with some components of the cell (lipids, proteins or DNA) and in so doing, they interfere with the cell's ability to function normally. Although, the body naturally produces free radicals, it also has a means to defend against its

harmful effects. Antioxidants which include some enzymes, are chemical substances found in the biological system that act on free radicals. Antioxidant enzymes work in several ways; they may reduce the energy of the free radical or give up some of their electrons for its use, thereby causing it to become stable. Antioxidant enzymes may also stop the free radical from forming in the first place. Similarly, they may also interrupt an oxidizing chain reaction to minimize the damage caused by free radicals. The main function of antioxidant enzymes is neutralizing free radicals produced in the body.

The human body produces several types of antioxidant enzymes which include superoxide dismutase (SOD), catalase, and glutathione peroxidase. These antioxidant enzymes neutralize many types of disease-causing free radicals, ridding the body of their harmful effects. Supplements of these antioxidant enzymes are also available. Usually they are for oral administration in the form of pills or capsules. However, the absorption of antioxidant enzymes in supplement form is minimal at best. A better way would be to supplement the body with the “building blocks” required in order for our body to manufacture its own SOD, catalase, glutathione peroxidase, and other such antioxidant enzymes. The building block nutrients of antioxidant enzymes include the minerals manganese, zinc, and copper for SOD and selenium for glutathione peroxidase. Hence this intervention programme has been explored by many researchers in order to ameliorate the toxic injury from pesticide exposure. In addition to antioxidant enzymes, many vitamins and minerals may also have antioxidant properties. These include vitamins A, C, E and nutrients such as lutein, lycopene, vitamin B₂, coenzyme Q₁₀, and cysteine. Herbs, such as bilberry, turmeric (curcumin), grape seed or pine bark extracts, and ginkgo can also provide powerful antioxidant protection for the body.

8. Non enzyme antioxidants

There are different types of antioxidants available in nature and their potential to fight free radical attacks has been widely exploited. Antioxidants from our diet appear to be of great importance in controlling damage by free radicals. Each nutrient is unique in terms of its structure and antioxidant function. Therefore, supplementation of these antioxidants in some cases of pesticide poisoning has proven to be of immense importance.

Vitamin E is actually a generic term that refers to all entities (eight found so far) that exhibit biological activity of the isomer tocopherol. Alpha-tocopherol, the most widely available isomer, has the highest biopotency, or strongest effect in the body. Because it is fat-soluble, alpha-tocopherol is in a unique position to safeguard cell membranes from damage by free radicals. Alpha-tocopherol also protects the fats in low-density lipoproteins from oxidation.

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. As such, it scavenges free radicals that are in an aqueous environment. Vitamin C works synergistically with vitamin E to quench free radicals. Vitamin C also regenerates the reduced (stable) form of vitamin E.

Beta-carotene, also a water-soluble vitamin, is the most widely studied of the 600 carotenoids identified till date. It is thought to be the best quencher of singlet oxygen. Beta-carotene is also especially excellent at scavenging free radicals in low oxygen concentration.

Selenium is a trace element that is required in very small quantities, without which we may not survive. It forms the active site of several antioxidant enzymes including glutathione peroxidase.

Similar to selenium, the minerals manganese and zinc are trace elements that form an essential part of various antioxidant enzymes.

9. Antioxidant enzymes

The antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) serve as primary line of defense in destroying free radicals. SOD first reduces the radical superoxide (O_2^-) to form hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase and glutathione peroxidase (GPx) then work simultaneously with the protein glutathione to reduce hydrogen peroxide and ultimately produce water (H_2O). Glutathione (commonly abbreviated GSH) is a crucial conjugating agent in the body. This compound is a tripeptide, meaning that it is composed of three amino acids linked together. These amino acids and their abbreviations are glutamic acid (Glu), cysteine (Cys), and glycine (Gly). A glutathione conjugate may be excreted directly, although this is rare. More commonly, the GSH conjugate undergoes further biochemical reactions that produce mercapturic acids (compounds with N-acetylcysteine attached) or other species. Glutathione forms conjugates with a wide variety of xenobiotic species, including alkenes, alkyl epoxides (1,2-epoxyethylbenzene), arylepoxides (1,2-epoxynaphthalene), aromatic hydrocarbons, aromatic halides, alkyl halides (methyl iodide), and aromatic nitro compounds. The glutathione transferase enzymes required for the initial conjugation are widespread in the body. The importance of glutathione in reducing levels of toxic substances can be understood by considering that loss of H^+ from $-SH$ on glutathione leaves an electron-rich $-S^-$ group (nucleophile) that is highly attractive to electrophiles. Electrophiles are important toxic substances because of their tendencies to bind to nucleophilic biomolecules, including nucleic acids and proteins. Such binding can cause mutations (potentially cancer) and result in cell damage. Included among the toxic substances bound by glutathione are reactive intermediates produced in the metabolism of pesticides, including epoxides and free radicals (species with unpaired electrons).

10. Superoxide dismutase (SOD)

The enzymes involved in antioxidant reactions are the superoxide dismutases, glutathione peroxidase and catalases. Superoxide dismutase (SOD) catalyses the destruction (dismutation) of superoxide free radicals produced during oxidation of pyrethroid (Otitoju and Onwurah, 2007). These ions are believed to be responsible for lipid peroxidation and peroxidative haemolysis of erythrocytes. The action of SOD therefore results in the protection of the biological integrity of cells and tissues against the harmful effects of superoxide free radicals. To ameliorate the damage caused by the hydroxyl radicals formed from superoxide radical and hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. SOD is an important isoenzyme functioning as superoxide radicals' scavenger in the living organisms. It is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress (Otitoju 2005). The action of SOD therefore results in the protection of biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Olusi, 2000). In order to ameliorate the damage caused by the hydroxyl radicals and hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. SOD is an important isoenzyme functioning as superoxide radicals' scavengers in the living organisms. It is an important enzyme family in living cells for maintaining normal

physiological conditions and for coping with stress. The role of superoxide dismutase enzyme is to accelerate the dismutation of the toxic superoxide radical (O_2^-) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. It is known that pesticides may irritate macrophages in the lung thereby encouraging them to produce superoxide radical (O_2^-). Antioxidant enzymes are used by the organisms as natural endogenous protection against the generation of reactive oxygen species (Metwalli and El-megd, 2002; Otitoju and Onwurah, 2007). Superoxide dismutases are metalloenzymes scavengers, which destroy superoxide radicals by converting them into hydrogen peroxide and oxygen by dismutation reaction. SOD works in conjunction with two enzymes, glutathione peroxidase and catalase which converts hydrogen peroxide to water and oxygen.

In our previous work on the effect of permethrin on SOD in non-target organisms, our results showed a marked increase in SOD activity in the exposed groups (Otitoju, and Onwurah, 2007). This increase may be a coping strategy for the exposed groups which may be due to other factors such as age, concentration of the pesticide, sex, e.t.c. However, depletion of SOD activity during prolonged exposure was suggested to be due to the overwhelming influence of superoxide radicals or activated metabolites generated by the pesticide on the cell membrane of the exposed organisms. The overall effect of pesticide radicals is the increased production of free radicals in the system and the concomitant decrease in the antioxidant activity due to the utilization of the antioxidant enzymes to neutralize the free radicals generated. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible. The oxidative destruction of lipids is a destructive, self-perpetuating chain reaction, releasing malondialdehyde (MDA) as the end product.

11. Glutathione transferase (GST)

Pesticides are metabolised through oxidation and hydrolysis by esterases, including other reactions involving glutathione, demethylation and glucuronidation. The glutathione transferase reactions give products that are, in most cases, of low toxicity. Pesticides are mostly eliminated in the urine with lesser amounts in the faeces and expired air. Glutathione-S-transferases are a major family of detoxifying enzymes that catalyze the conjugation of GSH (as earlier stated) with electrophilic centers of lipophilic substrates, thereby increasing its solubility and aiding their excretion from the body. Increased level of GST in a challenged or exposed individual may indicate that the OP and carbamate pesticides are mainly metabolised in the liver and excreted as a conjugate of GSH. Glutathione is an ubiquitous tripeptide that plays a significant role in oxidation-reduction reactions, amino acid transport, detoxification of electrophiles and metals, metabolites of xenobiotics and many carcinogens. Glutathione (GSH) is an endogenous thiol antioxidant that has a multifaceted role in xenobiotic metabolism and is a first line of defense against oxidant-mediated cell injury (Palmeira, 1999). Studies in animal models suggest that many synthetic organophosphates and organochlorides such as endosulfan and chloryriphos modify the concentrations of GSH (Beebe *et al.*, 2003). Glutathione together with glutathione dependent systems, glutathione peroxidase (GSH-Px), glutathione-S-transferase, catalase, and superoxide dismutase efficiently scavenge toxic free radicals.

12. Other antioxidants

In addition to enzymes, vitamins, and minerals, there appear to be many other nutrients (as mentioned in section 2), and compounds that have antioxidant properties. Among them is coenzyme Q₁₀ (CoQ₁₀, or ubiquinone), which is essential to energy production and can also protect the body from destructive free radicals. Also, uric acid, a product of DNA metabolism, has become increasingly recognized as an important antioxidant. Additionally, substances in plants called phytochemicals are being investigated for their antioxidant activity and health-promoting potential.

13. Mechanisms of biomarker action

A critical aspect of toxicological chemistry is that which deals with the biochemical mechanisms and reactions by which pesticides or related xenobiotic compounds and their metabolites interact with biomolecules to cause an adverse toxicological effect. In order to be detected or cause a toxic response, pesticides if introduced into an organism directly or indirectly, would react before reaching a target or receptor. However, when “reactive pesticides” are produced metabolically, it may be in a location where they can further interact with a biomolecule, membrane, or tissue to cause a toxic or biomarker response. Metabolically reactive pesticides generally fall into the following four categories:

- **Electrophilic species** that are positively charged or have a partial positive charge and therefore a tendency to bond to electron-rich atoms and functional groups, particularly N, O, and S, that abound on nucleic acids and proteins (including proteinaceous enzymes), which are commonly affected by toxic substances.
- **Nucleophilic species** that are negatively charged or partially so and have a tendency to bind with electron-deficient targets. These are much less common toxicants than electrophilic species, but include agents such as CO, formed metabolically by loss of halogen and oxidation of dihalomethane compounds or cyanide, CN⁻, produced by the metabolic breakdown of acrylonitrile, a biochemically reactive organic compound containing both a -CN group and a reactive C=C bond.
- **Free radicals** that consist of neutral or ionic species that have unpaired electrons. Free radicals include the superoxide anion radical O₂⁻, produced by adding an electron to O₂, and the hydroxyl radical, HO[·], produced by splitting (haemolytic cleavage) of the H₂O₂ molecule. These species can react with larger molecules to generate other free radical species. Electron transfer from cytochrome P-450 enzyme to a pesticide during its oxidation can produce the reactive, damaging free radical.
- **Redox-reactive** reagents that bring about harmful oxidation-reduction reactions. An example is the generation from nitrite esters of nitrite ion, NO₂⁻, which causes oxidation of Fe²⁺ in haemoglobin to Fe³⁺, producing methaemoglobin, which does not transport oxygen in blood. In understanding the kinds of processes by which some pesticides harm an organism, it is important to understand the concept of receptors. Here a **receptor** is taken to mean a biochemical entity that interacts with a pesticide to produce some sort of toxic effect. Generally receptors are macromolecules, such as proteins, nucleic acids, or phospholipids of cell membranes, inside or on the surface of cells. In the context of pesticide or its metabolite-receptor interactions, the substance that interacts with a receptor is called a **ligand**. Whereas an enzyme generally alters a substrate chemically (such as by hydrolysis), a toxicant does not usually change the

chemical nature of a receptor other than binding to it. In considering the interactions between the pesticide and the receptor, it may be assumed that the receptor normally binds to some endogenous substance, causing a normal effect, such as a nerve impulse. In some cases, the toxicant may activate the receptor, causing an effect similar to that of the endogenous ligand, but different enough in degree that some adverse effect results. Another possibility is that the toxicant binds to a receptor site, preventing an endogenous ligand from binding; this is known as an **antagonist action**. Yet another possibility is for the toxicant to bind to a site different from, but close enough to, the normal binding site to interfere with the binding of an endogenous substance. As a final possibility, the receptor may not have any endogenous ligands, but being bound by a toxicant nevertheless has some sort of effect.

Some pesticides can also exert their effect by interfering with enzyme activity. Enzymes are extremely important because they must function properly to enable essential metabolic processes to occur in cells. Substances that interfere with the proper action of enzymes obviously have the potential to be toxic. Many xenobiotics or pesticides that adversely affect enzymes are **enzyme inhibitors**, and thus they slow down or stop enzymes from performing their normal functions as biochemical catalysts. Stimulation of the body to make enzymes that serve particular purposes, a process called **enzyme induction**, is also important in toxicology. The body contains numerous endogenous enzyme inhibitors that serve to control enzyme catalyzed processes. When a toxicant acts as an enzyme inhibitor, however, an adverse effect usually results.

The covalent bonding of organic pesticides to enzymes can cause enzyme inhibition. Such bonding occurs most commonly through hydroxyl (-OH) groups on enzyme active sites. Covalent bonding of some pesticides is one of the major ways in which acetylcholinesterase (enzyme) crucial to the function of nerve impulses can be inhibited. An organophosphate compound, such as the nerve gas compound diisopropylphosphorfluoridate (a reactant), may bind to acetylcholinesterase, thereby inhibiting the enzyme.

14. Conclusion

Measurement of several biomarkers in albino rats at the same time for pesticides in the environment is of great importance. This approach has been advocated by several researchers who worked on different environmental toxicants (Romeo *et al.* 2003; Cajaraville *et al.* 2000). Since GST and SOD can be induced or inhibited in albino rats exposed to some pesticides, they can be used as biomarkers in risk assessment in a given human population. In fact increased levels/activities of glutathione S-transferase (GST) and superoxide dismutase (SOD) were observed in albino rats exposed to pyrethroid. Oxidative stress observed during pesticide metabolism in albino rats results in free radical and reactive oxygen species (ROS) formation. The consequence of the above is oxidation of cellular components such as membrane lipids, protein and DNA (biochemical markers), leading to adverse health effects and diseases. Based on the above, defense system against oxidative, (stress enhanced by the pesticide metabolism in rats), serves as the physiological endpoint parameters of exposure and they included superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase. Expanding the repertoire of these biomarkers of pesticide exposure and employing their multiple combinations in well-designed study protocols will provide critical tools in the evaluation of pesticide safety and

design of appropriate measures to minimize adverse exposures. Therefore, the combination of in vitro and animal data will give the best picture of biomarkers' performance.

15. References

- Anwar, W. A. (1997). Biomarkers of Human Exposure to Pesticides. *Environmental Health Perspectives* 105(4): 801-806
- Beeby, A (1993). Measuring the effect of pollution. In "Applying Ecology" Chapman and Hall, London, New York.
- Cajaraville, M.P., Bebianno, M.J., Biasco, J., Porte, C., Sarasquete, C., Viarengo, O. (2000). The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. *Sci. Total Environ.* 247, 295 – 311.
- Hagstrum, D. W and Subramanyam, B. H (2006). *Fundamentals of Stored-Product Entomology*. American Association of Cereal Chemists International, St. Paul, Minnesota
- Hulka, B.S. (1988). Biological markers. *Am J. Epidemiol.* 136, 200 -209.
- Mathews, P.J. (1980). Toxicology for water scientists. *J. Environ. Mgmt.* 11,(1), 1-14.
- Metwalli, A.M. and El-Magd. (2002). Skin diseases and enzymatic antioxidant activity among workers exposed to pesticides. *Eastern Mediterranean Health J.*, 8: 368 - 373.
- National Research Council (NRC), (1992), *Environmental Neurotoxicity* National Academy Press, Washington, DC
- Olusi, S.O. (2002). Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotective enzymes in humans. *International J. Obesity*, 26:1159-1164.
- Otitaju, O. (2005). Sub-chronic effect of Rambo insect powder contaminated diet on superoxide dismutase (SOD) activity in Wistar albino rats. *Biochemistri*.17 (2): 157-163
- Otitaju, O. and Onwurah, I.N.E. (2007). Glutathione S-transferase (GST) activity as a biomarker in ecological risk assessment of pesticide contaminated environment. *African J. Biotech.* 6 (12):1455-1459.
- Palmeira, C. M. (1999). Herbicide-induced mitochondrial and cellular toxicity: A review of paraquat, dinoseb and 2,4-D effects. *Toxic Substances Mechanisms*, 18: 187 – 204.
- Romeo, M., Hoarau, P., Garello, G., Gnassia-Barelli, M., Girard, J.P. (2003). Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. *Environ. Pollut* 122, 369 – 379.
- Schultz, P., and Mazzuckelli (1991). Validation of biological markers for quantitative risk assessment. *Environmental Health Perspective* 90, 239 – 246.
- US Environmental Protection Agency US EPA (2001). *Diazinon Revised Risk Assessment and Agreement with Registrants*, Office of Pesticide Programs.
- Ware G.W & Whitacre D.M (2004). *The Pesticide Book*. 6th ed. Meister Media Worldwide, Willoughby, Ohio. pp 496
- Ware, G.W (2000). *The Pesticide Book*. 5th Ed. Fresno, CA: Thompson Publications. pp 17.

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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