PESTICIDES MECHANISMS OF ACTION IN LIVING ORGANISMS

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Abstract. Pollution is a phenomenon, which leads to ecological disequilibrium (alteration of biotic and abiotic) and may produce dangerous waste. Epidemiological studies, evaluate the relationship between the pollutants impacts over individual or collective risk and environmental factors. The rational use of pesticides in conjunction with other technologies may be justifiable in integrated pest management, the balance between benefits and effects being very complex. Pesticides are considered persistent pollutants, and may be classified according to chemical structure in the following main classes: organophosphates, carbamates, organochlorines, triazines, and pyrethroids. In this paper we present the mechanisms of action of the main pesticide classes in living organisms and especially in the human body. Organophosphate pesticides act on acetylcholinesterase, leading to development of cholinergic toxicity, because they decrease its enzymatic activity. The carbamate or phosphate pesticides inhibition of acetylcholinesterase, disrupts the equilibrium between acetylcholine synthesis and release on one hand and its hydrolysis on the other, and leads to its accumulation at synaptic level, with prolonged activation of cholinergic receptors. Organochlorine pesticides are highly lipophilic, and this property enhances their stability in living organisms and in the environment. They are largely stored in adipose tissue, a process called bioaccumulation, and this characteristic leads to the development of high toxicities in mammals. Triazines in high concentrations have been linked to increased cancer risk and incidence of birth defects. The pyrethroid insecticides acting on the sodium channels in the nerve membrane (neurotoxic), have high selectivity for insects, and do not have carcinogenic, mutagenic and teratogenic effects. Living organisms and humans are concurrently exposed to pesticides from more than one source, via the environment and food, and these may have a combined (synergistic or

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antagonistic) action, which can cause higher or lower toxic effects, in comparison with the situation of a single pesticide.

Keywords: organophosphate pesticides; organochlorine pesticides; triazine pesticides; pyrethrin pesticides; enzyme; action mechanism

1. Introduction

Our present society is facing severe environmental problems, at the local, regional and global level. Maintaining or improving of the quality of the environment may be materialized through environmental monitoring.

The monitoring activities must identify and describe the evolution of the environmental qualitative and quantitative parameters in relationship with the affecting factors.

Pollution is a phenomenon, which leads to ecological disequilibrium (alteration of soil, air, water and biota) and consists of the following elements (Haiduc, 1996): pollution sources, pollutants transport and pollutants target.

The pollution affects air (climatic changes, stratospheric ozone depletion, acidification), waters (eutrophication, oxygen reduction), foods (reduced quality and quantity of nutrients), biota (habitat depletion and deterioration, species threatening) and may produces dangerous wastes (Colbeck et al., 2004).

The human body is exposed to an enormous number of non-nutrient compounds from the environment, many of which could be toxic. Epidemiological studies, evaluate the relationship between pollutants impacts over individual or collective risk and environmental factors.

2. Pesticides

Pesticides are among the environmental pollutant agents, which kill unwanted living organisms (animals or plants). Usually a pest is considered any living organism, which is interfering in a negative way with the human activity.

The rational use of pesticides in conjunction with other technologies may be justifiable in integrated pest management, the balance between benefits and effects being very complex.

The intended effects of pesticide use are as follows (Coman and Draghici, 2004):

- the control of pests and vectors of diseases;
- the control of organisms that harm other human activities.

On one hand, at community, national and global scales, there are three main benefit domains of pesticide use (Cooper and Dobson, 2007):

- social: health and quality of life;
- economic: farm (agricultural) costs and profits;
- environmental: terrestrial, aquatic and air.

On the other hand, the presence of pesticides in non-target species (including humans) can induces some perturbations, dependent on their nature, and their interactions with functional molecules.

Pesticides are a public health risk, and their use is the subject of some controversies concerning "what is acceptable risk" or "how can we minimize the risk" (George and Shukla, 2011).

Pesticides are considered persistent pollutants, and may be classified according to their chemical structure in the following classes: organophosphates, carbamates, organochlorines, triazines, and pyrethroids.

2.1. ORGANOPHOSPHATE PESTICIDES

All organophosphate pesticides contain a central phosphorus (P) atom to which an oxygen or sulfur atom is doubly bound (Figure 1). Two methoxy or ethoxy groups are also singly bonded to the central P atom, while a longer more complex structure (Z) is singly bonded to phosphorus, usually by an oxygen or sulphur atom (Coman and Draghici, 2004; Costa, 2006).

The toxicity of organophosphates is determined by the electrophilic magnitude of the central phosphorus atom, the steric nature of the substituents R_1 and R_2, and by the strength of the bond P – Z, and it is influenced by groups that increase the lability of the P –Z bond, which is broken during the inhibition process (Tahara et al., 2005).

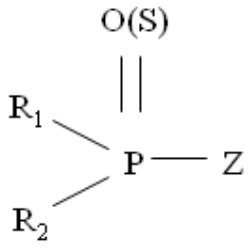

Figure 1. General structure of organophosphate pesticides

Organophosporus pesticides with p-CH_3–S–aromatic group or p-NO_2–aromatic group are the most toxic because these substituents enhance the electrophilicity of the P atom, by inductive and mesomeric effects. Mammals' metabolic transformation (biotransformation) of these groups, leads to magnification of the electrophilic character of P atom and increases the pesticide toxicity (p-CH_3–S group can be oxidized to p-CH_3–SO group or p-CH_3–SO_2 group).

Also, the toxicity of organophosphate pesticides decreases, with increasing chain length of R_1 and R_2 groups, because it decreases the electrophilic magnitude of the central phosphorus atom.

The primary target of organophosphate pesticides is acetylcholinesterase leading to development of cholinergic toxicity, because they decrease its enzymatic activity (Tahara et al., 2005; Badea et al., 2009; Singh et al., 2011).

Acetylcholinesterase is a serine enzyme with a hydroxymethyl group, present in the active site, which interacts with organophosphate pesticides to form an inactive complex (Figure 2).

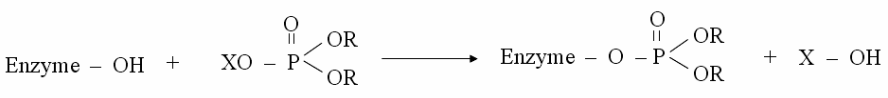

Figure 2. Representation of biochemical interaction between organophosphates and acetylcholinesterase (Enzyme – OH)

The physiological role of acetylcholinesterase is to catalyze the hydrolysis of acetylcholine, which is a major neurotransmitter in the central or peripheral nervous systems (Figure 3). Acetylcholinesterase is a key enzyme responsible for terminating the nervous impulse.

Acetylcholinesterase inhibition causes acetylcholine accumulation in the synaptic cleft and the postsynaptic membrane is continuously stimulated. Lack of coordination of the neuromuscular system is a direct result of this mechanism of action , and the final response can be death (Singh and Singh, 2000; Aygun et al., 2002).

Acetylcholinesterase inhibition can also alter lymphocytic activity, and organophosphates have been associated with the development of immunological disorders (Mahajan et al., 2006; Fabry et al., 2008;) or cancers (Carozza et al., 2009).

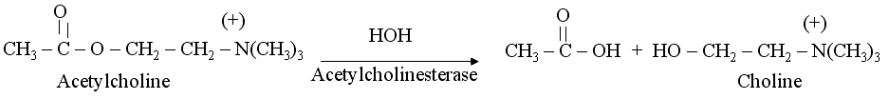

Figure 3. Catalytical activity of acetylcholinesterase

Organophosphate pesticides are weak inhibitors of acetylcholinesterase when a sulfur atom is bound to phosphorus (P = S) in the general structure (Sanchez et al., 2012).

In phase I biotransformation processes, cytochromes P450 (CYP_{450}) act as monooxigenases on organophosphate pesticides (P = S) and they are transformed by desulfurization, in the corresponding oxon analogs (P = O). Metabolic desulfurization process of organophosphorus pesticides is considered bioactivation (Costa, 2006; Badea et al., 2009), because oxon compounds are up to 1000 times stronger inhibitors of acetylcholinesterase, than the parent compounds (Figure 4).

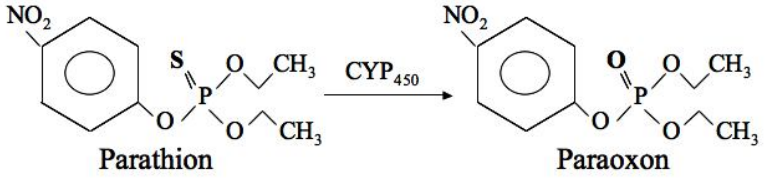

Figure 4. Biotransformation of parathion to paraoxon (bioactivation)

Organophosphates may also influence homeostasis of liver, brain and muscle where are glycogenesis (glycogen synthesis from glucose), gluconeogenesis (synthesis of glucose from nonzaharidic organic molecules), glycogenolysis (breakdown of glycogen to glucose) and glycolysis (glucose transformation to two molecules of pyruvic acid or lactic acid) are taking place, through pancreas control of glucose homeostasis, and by pancreatic control of insulin and glucagon secretion (Rahimi and Abdollahi, 2007).

Exposure to organophosphate pesticides (dimethoate, acephate, malathion, dichlorvos) induces hyperglycemia through decreased hexokinase activity in favor of glycogen storage activation (increase glycogen synthase activity) and in detriment of glycolysis (Rezg et al., 2006).

The induction of stress (the response to every situation which threatens homeostasis) or oxidative stress (alteration of the enzymes associated with antioxidant defenses) are the molecular mechanisms activated in pesticide

toxicity, especially in presence of organophosphate pesticides (Ranjbar et al., 2005).

Organophosphate pesticides induce reactive oxygen species generation and reactive nitrogen species generation, which contribute to the pathogenesis of acute pancreatitis through destruction of β-cells in diabetes and development of autoimmune diabetes (Rahimi and Abdollahi, 2007). Organophosphates are the most commonly used pesticides and are heavily used in agriculture and in urban settings as insecticides. Because of this, most of the population has been exposed to organophosphate pesticides in homes, outdoors, in workplaces, or through vegetal and animal foods consumption and in most of the subchronic or chronic disease cases there is no clear etiology because physicians cannot establish a direct cause-effect relationship between those and the pesticide toxicity.

2.2. CARBAMATE PESTICIDES

The carbamate pesticides are derivatives of carbamic acid, where one of the hydrogen atoms attached to nitrogen is replaced by a longer organic substituent (Fig.5).

The carbamate insecticides inhibit acetylcholinesterase, like the organophosphate insecticides (the same inhibition mechanism). Organophosphate and carbamate pesticides are primarily recognized by their acetylcholinesterase inhibition (Ferrari et al., 2007; Badea et al., 2008).

H_2NCOOH (a) R – NH – COOH (b)

Figure 5. General structure of carbamic acid (a) and carbamate pesticides (b)

The carbamate or phosphate pesticides inhibition of acetylcholinesterase, disrupts the equilibrium between acetylcholine synthesis and release on one hand and its hydrolysis on the other, and leads to its accumulation at synaptic level, with prolonged activation of cholinergic receptors (Pope et al., 2005).

Compared to organophosphate pesticides, the carbamate pesticides action is reversible, shorter in duration and milder in intensity.

2.3. HALOGENATED PESTICIDES

Halogenated compounds (organochlorine pesticides) are highly lipophilic, and this property enhances their stability in living organisms and in the environment. They are largely stored in adipose tissue, a process called

bioaccumulation, and this characteristic leads to the development of high toxicities in mammals.

The toxicity of organochlorine pesticides is determined by their structure, the spatial arrangement of their atoms (symmetry and asymmetry), and the nature of the substituents (Kaushik and Kaushik, 2007; Duarte et al., 2009).

The main characteristics of organochlorine insecticides are:

- preponderantly absorbed via the digestive system;
- accumulate in lipid rich tissues (adipose tissue, liver, milk, brain);
- interact with membrane lipoproteins (staple of the nerve membranes) causing the distortion of the nerve impulse transmission;
- adverse health effects associated with organochlorine pesticide exposure may be: neurological deficiency, respiratory problems, dermal damage, memory disorders and cancer.

DDT (dichlorodiphenyltrichloroethane) is a penta chloro aromatic compound, which acts on enzyme systems or nervous tissue, and is highly insecticidal (Figure 6).

DDT was the first widely used synthetic pesticide and the first pesticide used as insecticide in 1939 as an organic compound with extreme persistence, stability and low cost (Kaushik and Kaushik, 2007).

The use of DDT has been credited for the eradication of malaria in Europe and the United States and for revolutionizing agricultural production, but it has been associated with numerous pathologies in many animal populations (Beard, 2006).

In the developed countries DDT use has been restricted and continues to be used only as vector control.

DDT exerts its toxicity by binding to lipoproteins in the nerve cell membrane, disturbing the ionic homeostasis, especially the sodium/potassium balance across this membrane.

Also, DDT and its analogues can act through other mechanisms in the human body:

- interact with the neuron membrane;
- alter the transmission of the nervous impulses;
- alter the mitochondrial phosphorylation (cell energy).

The highly chlorinated cyclic hydrocarbons (aldrin, dieldrin, heptachlor, endrin, alodan, chlordane) acts as lindane or DDT, to produce convulsivant actions (cyclodiene insecticides).

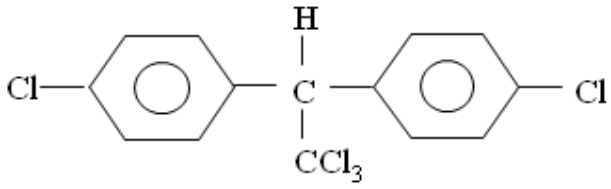

Figure 6. Structure of DDT

The presence of the double bonds in chlorinated cyclic hydrocarbons, increases pesticide toxicity, whereas presence of epoxide group in these compounds, decreased pesticide toxicity (Kanthasamy et al., 2005; Kaushik and Kaushik, 2007).

Aldrin is a hexachlorinated cyclodiene and resembles the dieldrin structure, which possess a stable epoxide ring (Figure 7). Dieldrin is a highly lipophilic compound and this property enhances its stability in the environment and in living organisms.

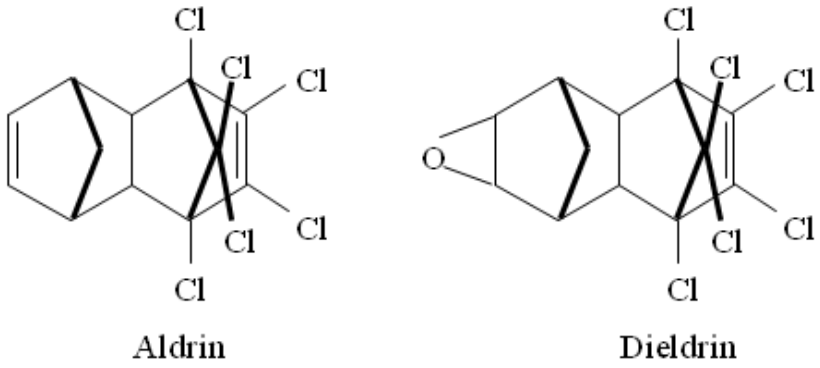

Figure 7. Structures of aldrin and dieldrin

Dieldrin is an organochlorinated pesticide, and it is classified as chlorinated cyclodiene with an unusually stable epoxide ring compared to other compounds.

In living organisms, dieldrin induces reactive oxygen species (ROS) production and as a result can cause (Kanthasamy et al., 2005; Rattan, 2010):

- a decrease of dopamine level, altering the function of dopamine transporters;

- perturbation of mitochondrial membrane potential, altering mitochondrial membrane perme-ability;
- the release of cytochrome c from inner mitochondrial membrane into the cytosol, where it activates caspases;
- caspase mediate hydrolytic cleavage and activation of protein kinase C, which acts on caspases by positive feedback (amplification of the apoptotic process);
- activated protein kinase C induced DNA fragmentation, which contributes to apoptotic cell death.

Exposure to chlorinated pesticides has been associated with some pathological states: non-Hodgkin's lymphoma, aplastic anemia, multiple myeloma, leukemia (Carozza et al., 2009).

2.4. TRIAZINE PESTICIDES

Triazines are moderately water-soluble herbicides (difficult to remove from potable water) and their chemical structure is based on an aromatic ring with three nitrogen atoms in alternating positions. Triazine derivatives possess some substituents (chlorine, amino groups) on the aromatic carbons (Figure 8).

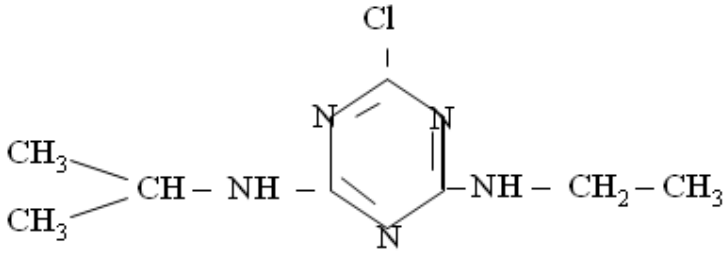

Figure 8. Structure of atrazine

The best-known member of the triazines is atrazine, which is not very acutely toxic, but, in high concentrations, has been linked to increased risk of developing cancer and an increased incidence of birth defects (Reffstrup, 2010).

2.5. PYRETHROID PESTICIDES

Pyrethroids are derivatives of natural pyrethrins and most of them contain cyclopropane carboxylic acid linked to alcohols through an ester or eter bond (Figure 9).

Addition of an α-cyano group to general structure of pyrethrins, confers an increase of one order of magnitude of insecticidal activity for target or neurotoxic activity for non-target species (Wolansky and Harrill, 2008).

Pyrethroids have 2-4 chiral carbons and the spatial conformation is determinant for their biological activity.

The pyrethroid insecticides acting on the sodium channels in the nerve membrane (neurotoxic), have high selectivity for insects, and do not have carcinogenic, mutagenic and teratogenic effects (Carle et al., 1982).

In humans the biotransformation of pyrethroids is rapid (ester hydrolysis or cytochrome P450 oxidation) and complete elimination from the body is realized in 2-8 days (detoxification).

A high dose of pyrethroids induces neurotoxicity in mammals (the non target species) in a similar manner to the effects it has in insects (the target species) for which pyrethroid pesticides possess a high selectivity. Compared to insects, mammals are 3 orders of magnitude less sensitive to pyrethroids and this characteristic supports the increasing substitution of other more toxic insecticide classes by these in order to decrease human occupational poisoning (Ray and Fry, 2006).

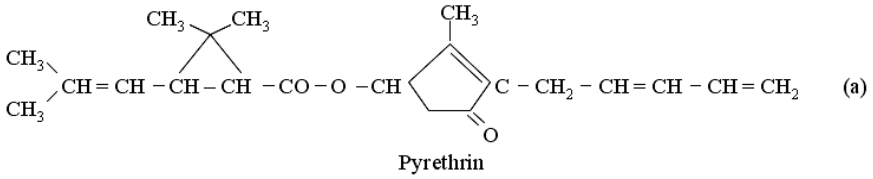

Figure 9. Structures of some natural (a) and synthetic pyrethroids (b)

Target systems for pyrethroids in mammals are: protein phosphorylation (cell energy), noradrenaline release, membrane depolarization (permeability), nicotinic receptors, lymphocyte proliferation (Clewell and Clewell, 2008).

2.6. ACTION MECHANISM THROUGH THE REACTIVE OXYGEN SPECIES

Paraquat (PQ) is a quaternary nitrogen herbicide, which is not absorbed from the gastrointestinal tract but is absorbed across the skin. Upon absorption, paraquat is little metabolized in the human body, and tends accumulates in the kidney and lung where it exerts its acute effects (Franco et al., 2010; Moretto and Colosio, 2011).

In humans, chronic paraquat exposure is a possible etiological factor for Parkinson's disease, which is a pathological state manifested by abnormalities of motor control: tremors, rigidity, and loss of reflexes.

In the human body, paraquat induces oxidative stress (production of reactive oxygen species) and alteration of the mitochondrial metabolism, manifested through changes in cell energy levels (Figure 10).

Living organisms and humans are concurrently exposed to pesticides, both via the environment and food, and these may have a combined (synergistic or antagonistic) action, which can causes higher or lower toxic effects, in comparison with the situation of single pesticide (Reffstrup et al., 2010).

The Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA) in the USA, the Health Council of the Netherlands, the Committee on Toxicity of Chemicals in Food, the Danish Veterinary and Food Administration, and other national or international organizations, recommended the introduction of the physiologically based toxicokinetic modelling (PBTK) as a tool in the risk assessment of pesticide mixtures.

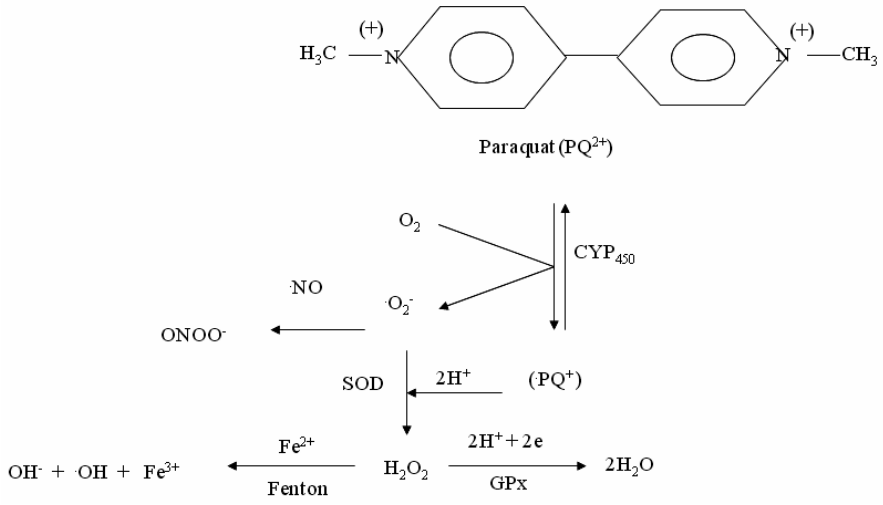

Figure 10. Paraquat ROS generation and oxidative stress

(SOD – superoxide dismutase; GPx – glutathione peroxidase)

These models require a large amount of data for construction, and once constructed and evaluated, they can be used for predicting the level of pesticides at the target site (ATSDR 2004; US EPA 2005; Clewell, and Clewell, 2008).

3. Conclusions

This paper presents some theoretical aspects of the pollutants (pesticides) and their action mechanisms in living organisms (mammals).

The carbamate or phosphate pesticides acetylcholinesterase inhibition, disrupt the equilibrium between acetylcholine synthesis, release and hydrolysis, and leads to their accumulation at synaptic levels, with prolonged activation of cholinergic receptors.

Organochlorine pesticides are highly lipophilic, and this property enhances their stability in living organisms and in the environment. This characteristic leads to the development of high toxicities in mammals.

Triazines can induces in high concentrations some disturbing links to cancer risk and incidence of birth defects.

The pyrethroid insecticides are high selectivity for insects, and are not carcinogenetic, mutagenetic and teratogenetic effects.

Living organisms and humans are concurrently exposed to pesticides and may have a combined (synergistic or antagonistic) action, which can causes higher or lower toxic effects, in comparison with the situation of single pesticide.

Pesticides are heavily used in agriculture and in urban as insecticides, and most of population has been contaminated to it in homes, outdoors, workplaces, or through vegetal and animal foods and most of subchronic or chronic diseases are not diagnosed, because cannot be established a direct cause-effect by physicians.

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