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Biological targets of cholinergic pesticides and possible use of alternative models for toxicity testing

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Abstract

The use of protection plant products for the control of pests in agriculture should be accompanied by a clear understanding of possible damages to human and environmental health. The mechanisms of action and the effects on developing organisms exerted by acute and chronic exposure to the main classes of cholinergic pesticides are reviewed.

Introduction

The present agricultural economy requests the use of plant protection products, in order to defend crops against infestations, and satisfy the request of a growing world population.
In the recent past (up to 1992) a great use was made for this purpose of organochlorine agents such as DDT, but recently these have been banned by almost all the Nations, cause of their persistence in the environment and in organisms, as persistence is responsible for dangerous accumulation and bioaccumulation of chemicals and their residuals. Thus, an increase has taken place in the use of organophosphate (OP) and carbamate (CB) pesticides, for their shorter lives.
OP is the name for the esters of phosphoric acid. OPs are at present the most diffused pesticides against a number of different infestations and crops, and are poured into the environment in the amount of million tons every season.
These compounds are easily synthesized, and their hemi life lasts from some days to some months in the Laboratory, at room temperature, but at temperatures lower than 0°C their activity is preserved for longer periods.
So, the persistence in the environment is not so easily predictable, because it depends on the temperature, rain, nature of the substrate, oxygen availability, etc.
The OPs primary target is the inhibition of acetylcholinesterase (EC. 3.1.1.7.) activity, but a number of secondary targets is known, e.g. butyrylcholinesterase (BChE, E.C. 3.1.1.8.), neuropathy target esterase (NTE) and carboxylesterase (E.C. 3.1.1.). In addition, OPs are known to block acetylcholine receptors, both muscarinic and nicotinic [1].
The OP toxicity is exerted cause of a stable link of the anionic portion of the molecule with a serine molecule in the catalytic site of AChE, that is at the bottom of a narrow "gorge" in the globular monomers. This plugs the gorge and prevents the ingression of ACh molecules.
The carbamate (CB) toxicity is due to the same mechanism, but carbamates are also competitive with ACh, and displace it from the catalytic site. Thus, acute toxicity of carbamates is stronger than the one of OP compounds (Fig. 1), but the CBs are less stable in the environment, so that chronic exposures are less dangerous.

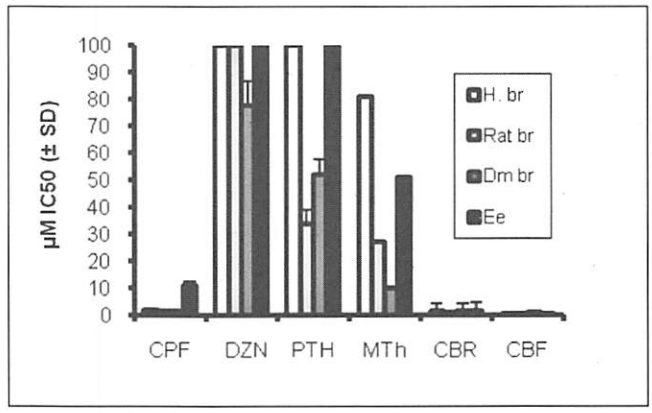

Figure 1. Comparison between IC50 dose of organophosphates (CPF, DZN, PTH and MTh) and carbamates (CBR and CBF). CPF = chlorpyrifos, DZN = diazinon; PTH = phentoin; MTh =malathion; CBR = carbaryl; CBF = carbofuran. Made by Rakonczay, in the frame of the EC project SENS-PESTI, QLK4, and reported by [2]. H br = human brain; Rat br = rat brain, Dm br = drosophila melanogaster brain, Ee = electric eel

A third class of cholinergic insecticides (neonicotin oids), act on the neuromuscular system of insects, with lower toxicity to mammals, because they affect a molecular form of nicotinic ACh receptor, which is typical of insects. The mode of action of neonicotinoids is similar to the natural insecticide nicotine, that like ACh activates the response of nicotinic ACh receptors, but is not cleaved by ChEs.
The main concern for the use of these insecticides is due to a possible connection to honey bee Colony Collapse Disorders (CCD) and generally for the disappearance of pollinator insects. According to what is reported in the

literature, no damage may be exerted on man, cause of the specific binding to insect receptors, but recent data may suggest a certain caution. Preliminary experiments show competition between Imidachloprid and α-BuTx, a snake venom from *Bungarus multicinctus*, which selectively binds to the α-7 subunit of the mammalian nicotinic receptor [3]. In addition, a fourth class of neurotoxic pesticides is represented by Pyrethroids, that exert their action by locking the Na^+ voltage-gated channels responsible for impulse conduction along the axons. These are not specifically cholinergic, but ultimately they can affect ACh release at the nerve endings.

Human diseases possibly related to occupational exposure

The exposure to acute doses of the contaminants is generally caused by accidents, or suicide intents. The effects of acute intoxication are mainly exerted on the nervous system, through the hyper-activation of receptors, causing peripheral nervous symptoms, also called cholinergic crisis, up to death [4]. The symptoms are due to the fact that AChE failure causes an increment of ACh at the receptor sites, with consequent block to muscarinic receptors (cardiac arrhythmia, salivation, lacrimation, hypotension, respiratory problems, headache, dizziness), and to nicotinic receptors, causing paralysis, muscular cramps, and tetanic contraction of muscles [5]. This crisis is sometimes followed by a more dangerous late onset of symptoms, such as asystole, which may appear after weeks, when the patient is released from the Hospital [6]. The effects of the acute intoxication are well known and classified as well as the first aid practice and antidotes, such as oxime and atropine [5, 7].

Chronic intoxication from low continuous or repeated doses

This is mainly caused by OP compounds, that, being liposoluble, pass through the cell membranes, the tissutal barriers, and can accumulate in the body lipids. One consequence is that they can reach intracellular receptors and act as endocrine interferents, or to overcome the threshold dose for effect (LOEL), reaching acute toxicity levels, e.g. in case of rapid weight loss.
At long term, nervous system disorders may occur: for instance, it has been reported that in areas where pesticides are spread, the incidence of certain neurodegenerative diseases is increased [8-10].
Respiratory effects may lead to aggravation of pre-existing conditions such as asthma [11], by bronco constriction [12]. Carbamates such as Carbaryl, may also cause morphologically deformed sperms.
Between 1991 and 1996, 3991 cases of occupational poisoning by agricultural pesticides were reported [13].
Domestic use of pesticides may cause symptoms that are similar or identical to those caused by other illnesses, so that chronic pesticide poisoning is often misdiagnosed.

Reliability of developing embryos as toxicity models

Neurotoxic pesticides have been demonstrated to affect embryonic development in invertebrates and vertebrates [2, 14-18]. Numerous case reports and case series present various combined severe congenital anomalies following occupational or accidental exposure of pregnant women to OP pesticides [19, 20]. This may be due to the fact that molecules related to the cholinergic system are present and functional in cells and tissues at the single events of development. These molecules are involved in the relatively slow cell-to-cell communication related to message exchanges during development and differentiation, before to be used in specialised structures such as synapses and neuromuscular junctions. The early developmental stages present a great ease to study the interaction with neurotoxic compounds, because the cells are excitable for a long period, huge respect to the cells of more complicated organisms, and the dyes identifying the ionic passages or presence of neurotransmitters and receptors may be visualised in the whole organism in one image. Thus the developmental events may supply a number of toxicity models for the study of the systemic cellular and tissutal effects of pesticides.

Cultured cells as alternative models.

In terms of gene expression analysis, cDNA microarray studies showed that the most statistically significant pathways affected were related to cellular death and cell proliferation [21]. In particular, Aluigi *et al.* [22] had evidence that the OP compounds may affect differentiation and cell proliferation/death of NTERA2-D1 cells (NT2). The NT2 cell line, which was derived from a human teratocarcinoma, exhibits properties that are characteristics of a committed neuronal precursor at an early stage of differentiation. By this model, the analysis of cell death could shed a light on the possible neurodegenerative risk represented by exposure to neurotoxic insecticides, as well as the effects on cell death and ChE expression in Sertoli cell cultures could represent an innovative and efficient biomarker of risk for fertility [23]. In mesenchyme stem cells obtained from bone surgery, it was found that micromolar concentrations of OPs and CBs had no effect on MSC survival or proliferation but limited MSC differentiation capacity by inhibiting osteogenic differentiation [24].
Thus it is evident that the cell type chosen for testing will help to understand the systemic activity of the contaminants and the related mechanisms.

Present problems and possible solutions

Epidemiological studies confirmed the previous described effects of chronic exposure [10], not only for agriculture operators but also for their relatives, as well as bystanders and consumers [25]. So, a new trend is emerging in consumers about the use of organic food. It is affirmed by several sources, that for pregnant women and children, the benefits are worth the higher price [26].
For this reason on one side, for operators it is needed a careful information and use of safety aids in the correct way. When used responsibly, pesticide products provide many benefits such as promoting affordable and abundant

food supplies. To ensure the safety of the environment and human health, pesticides are also heavily regulated by the Environmental Protection Agencies (EPA) of the different Nations. This includes evaluating whether pesticides pose an unreasonable risk to humans and the environment and requiring pesticide registrations when applicable. It is essential that all the information of the safe and proper use of the product is available and understood before a pesticide is used. To this aim, it is requested a great effort in the future for training of agriculture professionals, to provide them with a clear picture of risks and ways to avoid them.

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