[Frontiers in Bioscience 13, 1240-1249, January 1, 2008]

Neurotoxicity of pesticides: a brief review

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1. ABSTRACT

Pesticides are substances widely used to control unwanted pests such as insects, weeds, fungi and rodents. Most pesticides are not highly selective, and are also toxic to nontarget species, including humans. A number of pesticides can cause neurotoxicity. Insecticides, which kill insects by targeting their nervous system, have neurotoxic effect in mammals as well. This family of chemicals comprises the organophosphates, the carbamates, the pyrethroids, the organochlorines, and other compounds. Insecticides interfere with chemical neurotransmission or ion channels, and usually cause reversible neurotoxic effects, that could nevertheless be lethal. Some herbicides and fungicides have also been shown to possess neurotoxic properties. The effects of pesticides on the nervous system may be involved in their acute toxicity, as in case of most insecticides, or may contribute to chronic neurodegenerative disorders, most notably Parkinson's disease. This brief review highlights some of the main neurotoxic pesticides, their effects, and mechanisms of action.

2. INTRODUCTION

Pesticides are substances used for preventing, destroying, repelling or mitigating pests, intended as insects, rodents, weeds, and a host of other unwanted organisms (1). Ideally, the injurious action of pesticides would be highly specific for undesirable species; however, most pesticides are not highly selective, and are generally toxic to many nontarget species, including humans. Adverse health effects of pesticides in humans cover a variety of domains; some compounds may only exert some mild irritant effects in the skin, while others may affect liver or lung functions. Some are carcinogenic, other may cause reproductive toxicity or have endocrine disrupting properties. Several pesticides are neurotoxic. In particular, insecticides, which kill insects by disrupting their nervous system, exert neurotoxic effects in humans as well. A few compounds from other pesticide classes have also been associated with neurotoxic effects. Neurotoxicity can be manifested as severe signs and symptoms upon high acute exposure, or by more subtle effects upon chronic exposure to low doses. Exposure to pesticides is also thought to be a

risk factor in the development of neurodegenerative diseases, such as Parkinson's disease (2). This brief review summarizes the neurotoxic effects associated with exposure to certain pesticides. For a more comprehensive review of pesticide toxicology the reader is referred to other publications (1,41, 77-80).

3. PESTICIDES: CLASSIFICATION, USES, HUMAN POISONING

There are several different classes of pesticides, with different uses, mechanisms of action in target species, and toxic effects in nontarget organisms. Pesticides are usually classified according to their target species. The four major classes (and their target pests) are those of insecticides (insects), herbicides (weeds), fungicides (fungi, molds) and rodenticides (rodents), in addition to a number of "minor" classes such as, for example, acaricides (mites) or molluscides (snails, other mollusks). Within each class, several subclasses exist, with substantially different chemical and toxicological characteristics. For example, among herbicides, one can find bipyridyl compounds, triazines, chloroacetanilides, and several other chemicals.

Following a tremendous growth during the period 1950 – 1980, in the past twentyfive years the amount of pesticides used has stabilized. This is due to the utilization of new, more efficacious compounds, that require less active ingredient to obtain the same degree of pest control, and to the increasing popularity of organic farming. In the US, almost half of the pesticides used are herbicides, while in other countries, particularly Africa, Asia and Central America, there is also a substantial use of insecticides. Depending on climate, crops and pests, some countries may have a larger use of fungicides.

Exposure to pesticides can occur via the oral or dermal routes, or by inhalation. High oral doses, leading to severe poisoning and death, are usually the result of pesticide ingestion for suicidal intents, or of accidental ingestion. In contrast, chronic low doses are consumed by the general population as pesticide residues in food, or as contaminants in drinking water. Workers involved in the production, transport, mixing and loading, and application of pesticides, as well as in harvesting of pesticide-sprayed crops, are at highest risk for pesticide exposure. The dermal route is believed in this case to offer the greatest potential for exposure, with a minor contribution of the respiratory route when aerosols or aerial spraying are used. Pesticides are still involved in a large number of acute human poisonings. The World Health Organization (WHO) has estimated that there are around three million hospital admissions for pesticide poisoning each year, that result in around 220,000 deaths (3). Most occur in developing countries, particularly in Southeast Asia, and a large percentage is due to intentional ingestion for suicide purposes (4). Among pesticides, insecticides are the most acutely toxic. Herbicides, as a class, have generally moderate to low acute toxicity, one exception being paraquat. Fungicides vary in their acute toxicity, but this is usually low, while rodenticides are highly toxic to rats, but do not have the same toxicity in humans. Several studies in developing and developed countries indicate that insecticides (and particularly organophosphates), and the herbicide paraquat are most often responsible for human poisonings (5, 6).

4. NEUROTOXICITY: GENERAL CONCEPTS

Neurotoxicity can be defined as any adverse effect on the central or peripheral nervous system caused by chemical, biological or physical agents. A large number of chemicals have been shown to cause neurotoxicity; these include metals (e.g. lead), industrial chemicals (e.g. acrylamide), solvents (e.g. toluene), natural toxins (e.g. domoic acid), pharmaceutical drugs (e.g. doxorubicin), drugs of abuse (e.g. "ectasy") and pesticides (7). The nervous system is particularly sensitive to toxic insult, because of a number of intrinsic characteristics, such as dependence upon aerobic metabolism, the presence of axonal transport, or the process of neurotransmission (8). In addition, the developing nervous system, during which replication, migration, differentiation, myelination of neurons, and synapse formation occur, is believed to be even more susceptible to neurotoxic chemicals. This is compounded by the lack of a fully developed blood-brain-barrier. Indeed, several of the known neurotoxicants are primarily developmental neurotoxicants, and manifestations of neurotoxicity can be quantitatively and qualitatively different during development and in adulthood (e.g. in case of lead or ethanol).

From a general mechanistic perspective, neurotoxicants can be divided into four groups: those which cause neuronopathies, those which target the axon and cause axonopathies, those inducing myelinopathies, and those affecting neurotransmission (8). A number of chemicals can cause toxicity that results in the loss of neurons (neuronopathy), either by necrosis or by apoptosis. Such neuronal loss is irreversible, and may result in a global encephalopathy or, if only subpopulations of neurons are affected, in the loss of particular functions. Examples are MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which causes degeneration of dopaminergic neurons in the substantia nigra, resulting in Parkinson's disease-like symptoms (9), or trimethyltin, which targets hippocampal, amygdala and pyriform cortex neurons, resulting in cognitive impairment (10). In contrast, high doses of lead in adults may cause diffuse encephalopathy (11). Chemicals can cause neuronal cell death by a variety of mechanisms, including disruption of the cytoskeleton, induction of oxidative stress, calcium overload, or by damaging mitochondria. A large number of neurotoxic chemicals have as a primary target the axon, and cause axonopathies. The axon degenerates, and with it the myelin sheath surrounding the axon; however, the cell body remains intact. The chemical causes a "chemical transaction" of the axon at some point along its length, and the axon distal to the transaction, separated from the cell body, degenerates. The result is most often the clinical condition of peripheral neuropathy, in which sensations and motor strength are first inpaired in feet and hands (8). If insult is not severe, there is good potential for regeneration

Table 1. Major mechanisms and effects of neurotoxic insecticides in mammals

Insecticide class	Example	Mechanism	Effect
Organophosphate	Diazinon, Chlorpyrifos, Mipafox	Inhibition of AchE, Inhibition/aging of NTE (?)	Cholinergic syndrome, Peripheral axonopathy
Carbamate	Aldicarb Carbaryl	Inhibition of AChE	Cholinergic syndrome
Pyrethroid	Deltamethrin Allethrin	Prolonged opening of sodium channels	Hyperexcitability
Organochlorine	DDT Lindane	Prolonged opening of sodium channel, Inhibition of GABA- and voltage-dependent chloride channels	Hyperexcitability, tremors, Seizures
Formamidines	Amitraz	Activation of alpha 2-adrenoceptors	Hypotension

and recovery. Examples of chemicals causing axonopathies are the solvent n-hexane, and acrylamide (12, 13).

Other chemicals may target myelin, causing intramyelinic edema or demyelination. While neurons are structurally unaffected, their functions are altered. Triethyltin and hexachlophene are examples of chemicals that cause intramyelinic edema, leading to the formation of vacuoles creating a spongiosis in the brain. Lead, on the other hand, causes in adults a peripheral neuropathy with prominent segmental demyelination. Finally, there are neurotoxicants that interfere with neurotransmission (14). They can inhibit the release of neurotransmitter (e.g. botulinum toxin which inhibits acetylcholine release), act as agonist or antagonist as specific receptors (e.g. the marine neurotoxin domoic acid, which activates a subtype of glutamate receptors, or atropine which blocks muscarinic receptors), or interfere with signal transduction processes (e.g. lead, ethanol; 15). These effects are usually reversible, but nevertheless of toxicological relevance, as they may lead to severe acute toxicity or death.

5. NEUROTOXICITY OF INSECTICIDES

Insecticides play a relevant role in the control of insect pests. All of the chemical insecticides in use today are neurotoxicants, and act by poisoning the nervous systems of the target organisms. The taget sites for insecticides in insects are also found in mammals, hence insecticides are, for the most part, not species-selective with regard to targets of toxicity, and mammals, including humans, are highly sensitive to their toxicity (Table 1). As said, insecticides have higher acute toxicity toward nontarget species compared to other pesticides. There are several classes of insecticides; the most widely used are the cholinesterase inhibitors (organophosphates and carbamates), followed by the pyrethroids, and by other more recently developed compounds, such the neonicotinoids. The organochlorine compounds (e.g. DDT) were widely used until most of them were banned in the mid 1970s; however, some are still used in certain countries, and exposure of the general population still occurs, given their high environmental persistence.

5.1 Organophosphates and carbamates

Organophosphorus (OP) compounds were developed in the early 1940s, while carbamates were introduced as insecticides in the 1950s (16). Compounds of both classes have a common target of toxicity, the enzyme acetylcholinesterase (AChE), though they display different toxicological features. The general chemical structure of OPs consists in a phosphorus (P) atom bound with a double bond to an oxygen (O) or sulfur (S), and with three other single bonds to two alkoxy groups (OCH_3 or OC_2H_5), and with a so-called "leaving group" of different chemical nature. Most commonly used OP insecticides contain a sulfur bound to the phosphorus, and need to be metabolically bioactivated to exert their biological (or toxic) activity, as only compounds with a P = O moiety are effective inhibitors of AChE. This bioactivation consists in an oxidative desulfuration mediated by enzymes of the cytochrome P450 family (CYPs), which leads to the formation of an "oxon", or oxygen analog of the parent insecticide. All other biotrasformation reactions are detoxication reactions, as they lead to metabolites of lesser or no toxicity; some are mediated by CYPs, while others are hydrolytic reaction mediated by enzymes known as esterases (e.g. paraoxonase, carboxylesterase) (16,17).

OP insecticides have high acute toxicity, with oral LD_{50} values in rat often below 50 mg/kg, though for some compounds (e.g. malathion) toxicity is much lower, due to effective detoxication. The primary target for OPs is AChE, whose physiological role is that of hydrolyzing acetylcholine, a major neurotransmitter in the central and peripheral (autonomic and motor-somatic) nervous systems. Inhibition of AChE by OPs causes accumulation of acetylcholine at cholinergic synapses, with overstimulation of cholinergic receptors of the muscarinic and nicotinic type. As these receptors are localized in most organs of the body, a "cholinergic syndrome" ensues, which includes increased sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhea, tremors, muscular twitching, and various central nervous system effects. When death occurs, this is believed to be due to respiratory failure caused by inhibition of respiratory centers in the brainstem, bronchoconstriction and increased bronchial secretion, and flaccid paralysis of respiratory muscles (17, 18). At the molecular level, OPs with a P=O moiety phosphorylate a hydroxyl group on serine in the active (esteratic) site of the enzyme, thus impeding its action on the physiological substrate. Phosphorylated AChE is hydrolyzed by water at a very slow rate, but hydrolysis can be facilitated by certain chemicals (oximes) that are utilized in the treatment of OP poisoning. However, oximes are ineffective at reactivating phosphorylated AChE once the enzyme-inhibitor complex has "aged" . Aging consists of the loss (by nonenzymatic hydrolysis) of one of the two alkyl groups. When phosphorylated AChE has aged, the enzyme can be considered to be irreversibly inhibited, and the only means of replacing its activity is through synthesis of new enzyme, a process that may take days. Atropine, a cholinergic muscarinic antagonist, is the main antidote for OP poisoning; by blocking muscarinic receptors, it prevents the action of accumulating acetylcholine on these receptors.

As said, oximes, such as pralidoxime, are also used in the therapy of OP poisoning, and in some cases diazepam is also used to relieve anxiety or antagonize convulsions (18).

In addition to the acute cholinergic syndrome, OPs may also cause an intermediate syndrome, which is seen in 20-50% of acute OP poisoning cases (19). The syndrome develops a few days after the poisoning, during recovery from cholinergic manifestations, or in some cases, when patients are completely recovered from the initial cholinergic crisis. Prominent features of the intermediate syndrome are a marked weakness of respiratory, neck and proximal limb muscles. The intermediate syndrome is not a direct effect of AChE inhibition, and its precise underlying mechanisms are unknown, though it may result from nicotinic receptor desensitization due to prolonged cholinergic stimulation (18).

A third neurotoxic syndrome associated with exposure to a few OPs is the so-called organophosphate-induced delayed polyneuropathy (OPIDP). Signs and symptoms include tingling of the hands and feet, followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities, and then ataxia, which may occur 2-3 weeks after a single exposure, when signs of both the acute cholinergic and the intermediate syndromes have subsided (20, 21). OPIDP can be classified as a distal sensorimotor axonopathy, with the primary lesion in the distal part of the axon. OPIDP is not related to AChE inhibition, and indeed, one of the compounds involved in several epidemics of this neuropathy is tri-ortho-cresyl phosphate, which is a very poor AChE inhibitor. The putative target for OPIDP is an esterase, present in nerve tissues as well as other tissues, named neuropathy target esterase (NTE) (20, 22). Several OPs can phosphorylate NTE and inhibit its catalytic activity, in a fashion similar to what described for AChE. However, only OPs whose chemical structure leads to aging of phosphorylated NTE can cause OPIDP. Other compounds that inhibit NTE but cannot undergo the aging reaction, are not neuropathic, indicating that inhibition of NTE catalytic activity is not the mechanism of axonal degeneration. For OPIDP to be initiated, phosphorylation and subsequent aging of at least 70% of NTE is necessary, and this two-step process occurs within hours of poisoning. When the first clinical signs of OPIDP are evident some weeks later, NTE activity has recovered. The physiological functions(s) of NTE are unknown, and so is the series of events that takes place between phosphorylation and aging of NTE and axonal degeneration (21). Though several epidemics of OPIDP have occurred in the past, its occurrence in humans is now rare. Before commercialization, OPs must undergo specific neurotoxicity testing in the hen (one of the most sensitive species) to determine whether OPIDP is produced. Despite these tests, a few commercialized OPs have caused OPIDP in humans, mostly as a result of extremely high exposures in suicide attempts (21).

Two additional areas of concern regarding the neurotoxicity of OPs are possible long-term CNS effects in adults, and developmental neurotoxicity (23). Acute exposure to high doses of OPs may result, in some cases, in long-lasting adverse health effects in the CNS, as evidenced by animal and human studies (24, 25). In contrast, evidence of long-term CNS alterations in humans upon low, chronic exposure to OPs is contradictory, and current evidence does not fully support the existence of clinically significant neuropsychological effects, neuropsychiatric abnormalities, or peripheral nerve dysfunction in humans chronically exposed to low levels of OPs (26-28). Young animals, and presumably children, are more sensitive to the acute toxicity of OPs (23), and this increased sensitivity is believed to be due to lower detoxication abilities of young animals. In contrast, young animals are more resistant to OPIDP. Evidence is also accumulating which suggests that pre- and/or post-natal exposure to OPs may cause developmental neurotoxicity. OPs can disrupt various cellular processes (e.g. DNA replication, neuronal survival, neurite outgrowth), alter non-cholinergic pathways, induce oxidative stress, and cause various behavioral abnormalities (23, 29-32). Such effects are at times seen at dose levels that produce no cholinergic signs of toxicity. These findings have led to regulatory restrictions on the use of certain OPs in the home setting.

Carbamate insecticides derive from carbamic acid, and have different degrees of acute oral toxicity, ranging from moderate to low toxicity (carbaryl), to extremely high toxicity (e.g. aldicarb; LD_{50}=0.8 mg/kg). The mechanism of toxicity of carbamates is similar to that of OPs, as they also inhibit AChE. However,in case of carbamates inhibition is transient and rapidly reversible, since there is rapid reactivation of the carbamylated enzyme, and carbamylated AChE does not undergo the aging reaction. Nevertheless, the sign and symptoms of carbamate poisoning are the same observed following intoxication with OPs, and include miosis, urination, diarrhea, salivation, muscle fasciculation, and CNS effects. However, differently from OPs, acute intoxication by carbamates is generally resolved within a few hours. Carbamates are direct AChE inhibitors and do not require metabolic bioactivation. The treatment of carbamate intoxication relies on the use of the muscarinic antagonist atropine. Oximes have been shown to aggravate the toxicity of carbaryl, but may have beneficial effects in case of other carbamates, such as aldicarb (33). Carbamates can inhibit NTE, but since carbamylated NTE cannot age, they are thought to be unable to initiate OPIDP (21).

5.2. Pyrethroids

Pyrethroids are widely used as agricultural and household insecticides, in medicine for the topical treatment of scabies and head lice, and in tropical countries in soaked bed nets to prevent mosquito bites (34). They were developed in the 1970s by synthetic modifications of pyrethrins, natural compounds derived from the flowers of *Chrisanthenum cinerariaefolium*. Pyrethroids have high insecticidal potency and low mammalian toxicity. Indeed, despite their extensive use, there are relatively few reports of human poisonings (35). Pyrethroids are generally divided into two classes. Type I compounds produce in rats a syndrome consisting in marked behavioral arousal,

aggressive sparring, increased startle response, and fine body tremor progressing to whole-body tremor, and prostration (T syndrome). Type II compounds produce profuse salivation, coarse tremor progressing to choreoatetosis, and clonic seizure (CS syndrome) (34, 36). A key structural difference between type I and type II pyrethroids is the presence in the latter of a cyano group. However, certain pyrethroids elude such classification, as they produce a combination of the two syndromes. The mechanism of action of pyrethroids is the same in insects and in mammals, as they bind to the sodium channel and slow its activation, as well as the rate of inactivation, leading to a stable hyperexcitable state. Type I compounds prolong channel opening only long enough (<10 msec) to cause repetitive firing of action potential (37), while type II compounds hold the channels open for a longer period (>10 msec), so that the membrane potential ultimately becomes depolarized to the point at which generation of action potential is not possible (depolarization-dependent block) (36). These differences in the time of opening of sodium channels are believed to be at the basis of the differences observed between the T and CS syndromes (36). Type II pyrethroids can also inhibit $GABA_A$-gated chloride channels (38), albeit at higher concentrations than those sufficient to affect sodium channels. Type II pyrethroids also inhibit, at low concentrations, voltage-dependent chloride channels (39). These two effects are believed to mediate choreoatetosis, salivation, and seizures seen in the CS syndrome. Upon occupational exposure, the primary adverse effect resulting from dermal contact with pyrethroids is paresthesia (40). Symptoms include continuous tingling or pricking or, when more severe, burning. Paresthesia is presumably due to abnormal pyrethroid-induced repetitive activity in skin nerve terminals (36).

5.3. Organochlorine compounds

Organochlorine insecticides include DDT and its analogues; cyclodienes compounds, such as chlordane, aldrin or dieldrin; the hexachlorocyclohexanes, such as lindane; and cage-structured compounds such as chlordecone. From the 1940s to the 1970-80s, the organochlorine insecticides were widely used in agriculture, structure insect control, and malaria control programs. Their acute toxicity is moderate (less than that of organophosphates), but chronic exposure may be associated with adverse health effects particularly in the liver and the reproductive system. Primarily because of ecological considerations, these compounds have been banned in most countries in the past thirty years. Yet, because of their environmental persistence and high lipophilicity, exposure to these compounds continues, most notably through the diet. Furthermore, some, such as DDT, are being reintroduced in part of the world for malaria control.

The insecticidal activity of DDT [1,1,1-trichloro-2, 2-bis (4-chlorophenyl) ethane] was discovered in 1939. Technical DDT is a mixture of several isomers, with p,p' -DDT being responsible for the insecticidal activity. DDT has a moderate acute toxicity when given by the oral route, with an LD_{50} of about 250 mg/kg in rats. Acute exposure of rats to high doses of DDT causes motor unrest, increased frequency of spontaneous movements, hypersensitivity to external stimuli, followed by fine tremors, progressing to coarse tremors, and eventually tonic-clonic convulsions. Signs usually appear several hours after exposure, and death, usually due to respiratory failure, may follow after 24-72 hours (41). Signs and symptoms of poisoning are similar in most animals species. In humans, the earliest symptom of poisoning by DDT is hyperesthesia of the mouth and lower part of the face, followed by paresthesia of the same area and of the tongue. Dizziness, tremor of the extremities, confusion and vomiting follow, while convulsions occur only in severe poisoning. Both in insects and in mammals, DDT interferes with the sodium channels in the axonal membrane, by a mechanism similar to that of Type I pyrethroids (37). DDT prolongs the depolarizing (negative) afterpotential of the action potential, and this produces a period of increased neuronal excitability immediately after the spike phase. This, in turn, enhances the probability of repetitive firing, and the insurgence of a "train" of action potentials. Treatment for DDT poisoning focuses on the nervous system. In animals, phenytoin and calcium gluconate have been found to reduce DDT-induced tremors and mortality, respectively. In humans, in addition to decontamination and supportive treatment, diazepam or phenobarbital may be beneficial to control convulsions, if present.

Lindane is the γ isomer of benzene hexachloride (BHC), and the only isomer with insecticidal activity (42). Cyclodiene compounds include chlordane, dieldrin, aldrin, and heptachlor. These compounds were introduced in the early 1950s, and have experienced wide use before being banned in most countries due to their persistence and environmental and human health effects, one exception being lindane. Lindane and cyclodienes have moderate to high acute oral toxicity, and their primary target is the central nervous system. Unlike DDT, tremor is essentially absent, but convulsions are a prominent aspect of poisoning. These are due to the ability of these compounds to interfere with γ-aminobutyric acid (GABA) – mediated neurotransmission. Lindane and cyclodienes bind to a specific site (the picrotoxin site) on the chloride channel, thereby blocking its opening, and thus antagonizing the "inhibitory" action of GABA (43). Treatment of acute poisoning is symptomatic, and phenobarbital and diazepam can be used as anticonvulsants. Dieldrin exposure has been associated with Parkinson's disease. Elevated levels of this compound were found in post mortem brain from Parkinson's disease patients (44). Recent findings indicate that repeated dieldrin exposure can cause oxidative damage to the mouse nigrostriatal system (45), thus providing biological plausibility for its possible role as a risk factor in Parkinson's disease.

Chlordecone (Kepone) is another organochlorine insecticides no longer in use. The primary manifestation of its toxicity, in animals and in humans, is the presence of tremors (46). The exact mechanism of chlordecone neurotoxicity has not been elucidated, but it is believed to involve inhibition of ATPases (both Na^+ -K^+ and Mg^{++} ATPases), and ensuing inhibition of the uptake of catecholamines (47). In contrast to cyclodienes, chlordecone does not cause seizures.

5.4. Other insecticides

Several other classes of insecticides exist, that can have neurotoxic effects in nontarget species, and a few are discussed below. Formamidines, such as amitraz [N′-2,4-(dimethyl-phenyl)-N-N ((2,4-dimethylphenyl) imino) methyl-N-methanimidamide], are used in agriculture and in veterinary medicine as insecticides/acaricides (48). Their structures are closely related to the neurotransmitter norepinephrine. In invertebrates, these compounds exert their toxicity by activating an octopamine-dependent adenylate cyclase, while in mammals, where symptoms of poisoning are sympathomimetic in nature (49), α_2-adrenergic receptors are believed to be the target for formamidine toxicity. In vivo and in vitro studies have indeed shown that formamidines act as rather selective agonists at α_2–adrenergic receptors (50). In recent years, several cases of acute amitraz poisoning have been reported, particularly in Turkey, and most involved children (51). Signs and symptoms of poisoning mimicked those of α_2-adrenergic receptor agonists such as clonidine, and included nausea, hypotension, hyperglycemia, bradycardia and miosis, but no deaths were recorded. Though α_2-adrenoceptor antagonists such as yohimbine have proven useful as antidotes in animals (52), their usefulness in managing amitraz poisoning in humans has not been evaluated.

Rotenone is a rotenoid ester, present in the roots of the East Asian Derris plants, particularly *D. Elliptica.* Rotenone is used as an agricultural insecticide/acaricide, particularly in organic farming (53). The toxicity of rotenone in target and nontarget species is due to its ability to inhibit, at very low concentrations, the mitochondrial respiratory chain, by blocking electron transport at NADH-ubiquinone reductase, the energy conserving enzyme complex commonly known as Complex I. Poisoning symptoms include initial increased respiratory and cardiac rates, clonic and tonic spasms, and muscular depression, followed by respiratory depression. In the past few years, rotenone has received some attention because of its potential role in the etiology of Parkinson's disease. Repeated administration of rotenone to rats causes selective nigrostriatal degeneration and produces protein inclusions, similar to Lewy bodies, both hallmarks of Parkinson's disease (54). Rotenone may thus represent an useful experimental model, however, its role in the etiology of Parkinson's disease in the general population is still unproven (55).

Nicotine is an alkaloid extracted from the leaves of tobacco plants. It is a systemic insecticide effective toward a wide range of insects. Nicotine exerts its pharmacological and toxic effects in mammals and insects by activating nicotinic acetylcholine receptors. Interaction of nicotine with nicotinic receptors produces initial stimulation followed by protracted depolarization, which results in receptor paralysis. Nicotine has a high acute toxicity in vertebrates, with LD_{50}s usually below 50 mg/kg (56). Signs and symptoms of poisoning include nausea, vomiting, muscle weakness, respiratory effects, headache, lethargy, and tachycardia. By chemical modifications of nicotine and other nicotinic agonists, new classes of insecticides have been developed that are referred to as neonicotinoid (e.g. imidacloprid, nitenpyram) (57). The insecticidal activity of neonicotinoids is attributed to activation of nicotinic receptors, and their mammalian toxicity is similar to that of nicotine. However, in contrast to nicotine, neonicotinoids display a high selectivity and specificity toward insect nicotinic receptors. This favorable selectivity profile explains their commercial success, and they now account for 10-15% of the total insecticidal market (58).

6. NEUROTOXICITY OF HERBICIDES

Chemicals used as herbicides can kill or severely injure plants. As most mechanisms of herbicidal action involve biochemical pathways that are unique to plants, herbicides have lesser acute toxicity than insecticides, an exception being paraquat. Yet, various classes of herbicides have been associated with adverse effects ranging from carcinogenicity, target organ toxicity, or endocrine disruption. With regard to neurotoxicity, the widely used chlorophenoxy herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) has been associated , mostly in case reports, to a variety of neurological effects, ranging from peripheral neuropathy to behavioral alteration (59). However, chronic toxicity studies provide very limited evidence of neurotoxicity of 2,4-D (60).

Paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride) is the compound of most neurotoxicological interest.

It has one of the highest acute toxicity among herbicides, with an oral LD_{50} in rat of approximately 100 mg/kg, and even higher toxicity in guinea pigs, rabbits and monkeys. Upon absorption, independently of the route of exposure, paraquat accumulates primarily in the lung and secondarily in the kidney. The mechanism(s) by which paraquat is toxic to cells have been extensively investigated (61, 62). Paraquat can be reduced to form a free radical, which in the presence of oxygen, rapidly reoxidizes to the cation, with a concomitant production of superoxide anion ($O2^{-\bullet}$), a process known as redox cycling. The ensuing cytotoxicity is believed to be due to generation of superoxide anion and subsequently of hydroxy radicals, that would initiate lipid peroxidation, ultimately leading to cell death (62). Upon acute exposure to lethal doses of paraquat, mortality may occur 2-5 days after dosing. Since its introduction as an herbicide, there have been thousands of episodes of acute poisoning with paraquat in humans, with a very high mortality rate (63). Chronic paraquat exposure has been suggested as a possible etiological factor in the development of Parkinson's disease. The hypothesis arose by the structural similarity of paraquat to MPP^+ (1-methyl-4-phenylpyridinium ion), the toxic metabolite of MPTP. MPP^+ itself was initially developed as a possible herbicide, but was never commercialized. It has been argued that paraquat, being positively charged, cannot easily pass the blood-brain-barrier. Yet, animal studies have shown that paraquat can cause CNS effects, most notably a neurodegeneration of dopaminergic neurons (64). Paraquat may be transported into the brain by a neutral amino acid transporter, such as the system L carrier (LAT-1) (65). The

ability of paraquat to cause oxidative damage through a free radical mechanism may explain the selective vulnerability of dopaminergic neurons, which are per se more susceptible to oxidative damage (66). Though these findings in animals are compelling, there is no solid evidence that paraquat may be associated with Parkinson's disease in humans (55).

7. NEUROTOXICITY OF FUNGICIDES

Fungicides comprise a large number of compounds, with diverse chemical structures, extensively used to provide crop protection toward fungi and molds. Several fungicides are carcinogenic (e.g. captan), while others may have skin or eye irritant properties (e.g. chlorothalonil). With regard to neurotoxicity, compounds of interest are some organic metal compounds that are no longer used, and the dithiocarbamates. Several inorganic and organic metal compounds are, or have been, used as fungicides (67). Among these, organic mercury compounds, such as methylmercury, were used extensively as fungicides in the past for the prevention of seed-borne diseases in grains and cereals. Given their high neurotoxicity, and large episodes of human poisoning (68), their use has since been banned. Clinical manifestations of methylmercury neurotoxicity include parasthesia, ataxia, fatigue, hearing and vision loss, plasticity and tremors (69). In utero exposure is particularly deleterious, and widespread neuronal death and astrocytosis can be observed in such cases (70).

Dithiocarbamates are a group of fungicides that have been widely used since the 1940s to control fungal pathogens in a variety of crops. The nomenclature of many of these compounds arises from the metal cations with which they are associated; examples are Maneb (Mn), Ziram and Zineb (Zn) and Mancozeb (Mn and Zn) The dithiocarbamates have low acute toxicity, however, chronic exposure is associated with adverse effects that may be due to the dithiocarbamate acid or the metal moiety. Developmental toxicity and teratogenicity is observed with dithiocarbamates at maternally toxic doses. These effects are ascribed to an action of the metabolite ethylenthiourea (ETU) on the thyroid. A key concern with chemicals affecting thyroid functions, is their potential developmental neurotoxicity, given the essential role of thyroid hormones in brain development (71). There is also some evidence that dithiocarbamates may cause neurotoxicity by mechanisms not involving ETU. High doses of several of these compounds cause hindlimb paralysis, which is possibly related to the release of the carbon disulfide moiety (72). Chronic exposure to Maneb has been associated with Parkinsonism, which may be due to the manganese moiety, rather than the dithiocarbamate (73,74). Maneb has also been shown to produce nigrostriatal degeneration when given in combination with paraquat (75), and to directly affect dopaminergic neurons by inhibiting mitochondrial functions (76). The structure of dithiocarbamate fungicides resembles that of disulfiram, a compound used therapeutically to produce intolerance to alcohol, by virtue of its ability to inhibit aldehyde dehydrogenase. Interactions of dithiocarbamates with alcohol, leading to elevation in acetaldehyde levels, have been reported.

8. CONCLUSIONS AND PERSPECTIVES

This brief overview highlights the most relevant aspects of neurotoxicity associated with exposure to pesticides. As said, insecticides are most often responsible for neurotoxic effects in humans, as the nervous system represents their biological target in insects. In addition to implications for acute toxicity, exposure to pesticides raises concerns for possible long-term effects and developmental effects. Whether chronic exposure to low doses of certain pesticides may contribute to the etiology of some neurodegenerative diseases (most notably Parkinson's disease) and/or to other less defined behavioral alterations, remains a topic of public concern, and more research is needed. Furthermore, the possibility that developmental exposure to pesticides (both in utero and neonatally) may contribute to developmental disorders in children, such as attention deficit hyperactivity disorder, autism, or learning disabilities, needs to be further investigated. Finally, a most challenging endeavor would be that of ascertain whether developmental exposure to pesticides may result in "silent neurotoxicity", i.e. may cause nervous system damage that would be manifest as a clinical condition only later in life. For example, damage to nigrostriatal dopaminergic neurons early in life would be expected to result in clinical manifestations of Parkinson's disease as the individual ages, by adding the early insult to the normal age-related loss of neurons. This theoretical possibility needs to be investigated in experimental animal models.

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Key Words: Pesticides, Insecticides, Organophosphates, Neurotoxicity, Review

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