

## Review

# Cytotoxicity of chlorpyrifos and cypermethrin: The ameliorative effects of antioxidants

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**The present paper reviews the mechanism underlying chlorpyrifos and cypermethrin poisoning. It explains the mechanism of action of chlorpyrifos and cypermethrin and the role of oxidative stress. It sheds light on the interaction between chlorpyrifos and cypermethrin as observed in many cocktails of pesticide combinations today. It also explains the adverse health effects of pesticides and some antioxidants which may ameliorate their effects. Further research aimed at identifying more agents that may ameliorate chlorpyrifos and cypermethrin-induced toxicity should be carried out.**

**Key words:** Pesticides, chlorpyrifos, cypermethrin oxidative stress, antioxidant.

## INTRODUCTION

Pesticides are substances used for preventing and controlling pests, including vectors of human or animal diseases. They are used to control unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, or transport or marketing of food, agricultural commodities, wood and wood products, animal feedstuffs or substances, which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (WHO, 2002). Although they are beneficial in improving food production, reducing manpower farm needs and improving public health, pesticides adversely affect human and animal health and environmental sustainability. In the past few decades, the resistance of pests to conventional pesticides was on the increase. This has posed serious challenges to the farmers, public health workers, researchers, chemists and the general populace (Konradsen et al., 2003). Throughout the world, the challenges have led to the production or synthesis of

more potent pesticides with attendant side-effects (Coronado et al., 2004). Recently, the use of pesticide combinations to combat the menace of pesticide resistance has come to the fore. This however comes with its adversity, which is recently unfolding. One of the most popular insecticide combinations is the organophosphates and pyrethroids.

Organophosphates (OPs) are a group of insecticides discovered in 1938 by some German scientists. They were introduced as nerve poisons or chemical warfare agents during World War II (Echobichon, 1996). OPs are biodegradable with low toxicity, but, compared to the organochlorines, have adverse effect on fertilization, the liver (Hernandez et al., 2006) and central nervous system (Yang and Deng, 2007). They are used indiscriminately in large amounts and are predominantly involved in progressive environmental pollution. Recently, evidence of adverse health due to low-level exposure to OPs has begun to emerge (Peiris-John and Wiekremasinghe, 2008).

Pyrethroids are synthetic forms of pyrethrins (insecticides derived from *Chrysanthemum* plants extracts) (Soderlund, 2002). There are two types of pyrethroids that differ in chemical structure; the type I

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includes allethrin, tetramethrin, resmethrin, bioremethrin, and permethrin, while type II includes cypermethrin, cyfluthrin, cyphenothrin and deltamethrin. Both types I and II inhibit the nervous system of insects (Wang et al., 2006). Type I pyrethroids cause mainly hyperexcitation and fine tremors, while type II produces a more complex syndrome, including clonic seizures (Verschoyle and Aldridge, 1980). Type I pyrethroids modify the sodium channels in the closed state, whereas type II pyrethroids modify the open but not inactivated sodium channels (Soderlund, 2002).

Chlorpyrifos (CPF; 0,0-didsethyl0-3,5,6-trichloro-2-pyridylthiophosphate) is a broad spectrum OP pesticide used against a wide variety of insects, including fleas, wasps, bees, cockroaches and agricultural pests (Eaton et al., 2008). It is a chlorinated OP, synthesized by Dow Chemical Company in U.S.A., and introduced into the market in 1965. It is one of the most widely used OP insecticides, despite restrictions placed on some of its domestic applications by United States Environmental Protection Agency in 2000. CPF has been reported to affect brain development, resulting in altered synaptic development, and alteration in DNA and protein synthesis (Hoberman, 1998a). Behavioural aberrations following exposure to CPF occur in both young and adult rats (Schom et al., 1973; Ambali et al., 2010; Ambali and Ayo, 2011; Ambali and Aliyu, 2012).

### MECHANISM OF TOXICITY OF CPF

The principal mechanism of toxicity of CPF is due to its inhibition of acetylcholinesterase (AChE), resulting in the accumulation of the neurotransmitter, acetylcholine (ACh) at the nerve endings and the neuromuscular junctions (Eaton et al., 2008). However, toxicity of CPF also occurs at doses that do not inhibit AChE or long after its restoration (Pope et al., 1992). This means other mechanisms independent of AChE inhibition are involved in the induction of CPF neurotoxicity. Oxidative stress is one of the mechanisms that have been implicated in CPF-evoked neurotoxicity (Gulterkin et al., 2006; Ambali et al., 2010).

### CYPERMETHRIN

(R,S)alphacyano3phenoxybenzyl(RIS)cis,trans3(2,2dichlorovinyl)2,2dimethylcyclopropane-carboxylate (Kidd and James, 1991) like many other pyrethroids, has been known to cause adverse effects on the nervous system (Saha and Kavirajand, 2003). Chronic symptoms after exposure to pyrethroids include brain and locomotion disorders, polyneuropathy and immunosuppression, resembling the multiple chemical sensitivity syndrome (Pascual and Peris, 1992).

### Mechanism of action of cypermethrin

During metabolism, cypermethrin forms cyanohydrines, decomposing further to cyanides and aldehydes; substances that can induce production of reactive oxygen species (Leja-Szpak, 2004). An increase in reactive oxygen species/free-radical-mediated lipid peroxidation and increased cytosolic calcium concentration (which occurs due to direct effect of pyrethroids on calcium channels) may lead to cytotoxicity and genotoxicity in higher vertebrates during exposure (Kadous et al., 1994; Gassner et al., 1997; Kale et al., 1999).

Organophosphates and pyrethroids belong to the most often used group of insecticides. They are characterized by high insecticidal potency. Acute exposure causes serious adverse effects in humans and other mammals due mainly to neurotoxic action. In the case of OPs, the neurotoxicity is linked with the inhibition of AChE and, as a consequence, pathological retention of ACh in the synaptic gaps (Chiappa et al., 1995). Interaction of cypermethrin with sodium channels produces a hyperexcitable state, and is the major mechanism of its neurotoxicity (Ray, 2001).

### INTERACTION OF CHLOPYRIFOS AND CYPERMETHRIN

The interaction between OPs and pyrethroids is relatively well known, since formulations containing both classes of insecticides are available in the market. The non-reversible inhibition of esterases by OPs leads to slowing down the activity of enzymes responsible for cleavage of the ester bond in the pyrethroid molecule (Latuszynska et al., 2001). In normal conditions, the ester bond is quickly metabolized in mammals, the products of which are non-active and rapidly excreted from the body mainly through the urine. Blocking the hydrolysis of pyrethroids significantly reduces the metabolism of these pesticides, therefore, a stronger insecticidal effect of the OP-pyrethroid mixture is observed. From an economical and ecological point of view, such a combination enables reduction in the amount of each of the pesticides used, while keeping insecticidal efficiency at the same level (Timchalk et al., 2005).

The use of insecticide combinations in agricultural pest control and public health is on the increase due to the challenges posed by insecticide resistance to the existing chemicals. This, however, comes with its new challenges in terms of toxicity and environmental pollution. CPF is the most studied OP compound, primarily because of its large usage in crop protection and also its wide non-agricultural applications in households. Its metabolite, 3,5,6-trichloro-2-pyridinol is the most frequently found pesticide degradation product in urine of the general population, suggesting wide CPF exposure in humans

(Koch and Angerer, 2001). Cypermethrin, a synthetic pyrethroid insecticide, has been extensively used in the last two decades in many countries. Studies have shown that prolonged exposure to these contaminants causes chronic or persistent neurologic (Betarbet et al., 2000; Alavanja et al., 2004), immunosuppressive (Repetto and Baliga, 1996) and teratogenic effects, abortion and other reproductive failures (Bretveld et al., 2008). Pesticides may induce oxidative stress by generating increased free radicals and decreasing antioxidant levels and activities of free-radical scavenging enzymes (Sharma et al., 2005). Exposure to many types of environmental contaminants, including pesticides, enhances this oxidative damage by increasing the generation of free radicals and or by decreasing antioxidant potentials in the body (Wang et al., 2003).

## ADVERSE HEALTH EFFECTS OF PESTICIDES

### Acute cholinergic syndrome

OP compounds exert acute systemic toxicity by inhibiting the activities of the enzyme AChE through a process of phosphorylation. Pesticides bind to cholinesterase and block the hydrolysis of the choline and acetic acid at the post synaptic junctions, thus resulting in acetylcholine accumulation (Eaton et al., 2008). OP-induced neuronal symptoms are a consequence of axonal death. Following OP exposures, inhibition of neuronal enzymes called neuropathy target esterase occurs and many of them are irreversible (Abou-Donia, 2003).

### Intermediate syndrome

This consists of a sequence of neurological signs that appear 24 to 36 h after the acute cholinergic crises (Senanayake and Karalliede, 1987). In this form, most animals do not have obvious signs of acute syndrome, but instead they develop tetraparesis, mydriasis and ventroflexion of the neck several days post exposure (Yang and Deng, 2007). The intermediate syndrome is not a direct effect of AChE inhibition, and its precise underlying mechanisms are unknown (Eaton et al., 2008).

### Organophosphate induced delayed polyneuropathy

The organophosphate induced delayed polyneuropathy (OPIDP) is a neurodegenerative disorder, characterized by a delayed onset of ataxia and upper motor neurone spasticity as a result of a single or repeated exposure to OPs (Abou-Donia, 2003). It has been hypothesised that the hyperphosphorylation of cytoskeletal proteins results

in the destabilisation of microtubules and neurofilaments, leading to their aggregation and deregulation in the axon, which causes its degeneration (Abou-Donia, 2005). OP ester-induced delayed neurotoxicity is characterised by a motor-sensory deficit resulting from Wallerian-type degeneration of the axon, followed by demyelination of the central and peripheral nervous systems (Abu-Donia, 1981). Numbness, tingling sensation weakness and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years and in some cases residual impairment will remain (Barone et al., 2000).

### Organophosphate induced chronic neurotoxicity

The organophosphate induced chronic neurotoxicity (OPICN) which is distinct from both cholinergic and OPIDN is produced by exposure to large, acutely toxic or small subclinical doses of OP compounds (Jamal, 1997). Clinical signs which continue for prolonged times, ranging from weeks to years after exposure, consist of neurological and neurobehavioural abnormalities. Both the peripheral and central nervous systems are damaged, but with greater involvement of the latter (Abou-Donia, 2003). Within the brain, neuropathological lesions are observed in various regions, including the cortex, hippocampus and cerebellum. The lesions are characterized by neuronal cell death, resulting from early necrosis or delayed apoptosis (Abou-Donia, 2003). Neurological and neurobehavioural alterations are exacerbated by concurrent exposure to stress or to other chemicals that cause neuronal cell death or oxidative stress. since CNS injury predominates, improvement is slow and complete recovery is unlikely (Jamal, 1997).

## ORGANOPHOSPHATES AND OXIDATIVE STRESS

Oxidative stress is an important component of the mechanism of OP compound toxicity (Ambali et al., 2007; Suke et al., 2008). Several studies explained that oxidative stress is involved in OPs toxicity (Hsu et al., 2001; Prakasam et al., 2001; Vidyasagar et al., 2004; Shadnia et al., 2005). Repeated daily oral doses of pesticide in rats altered the biochemical parameters and antioxidant status (Manni, 2004). Several studies reveal that OPs induce oxidative stress, leading to generation of free radicals and alteration of antioxidant status (Bagchi, 1995; Abdhollahi, 2004). Toxic effects of pesticides on human beings are confirmed by the direct measurement of lipid peroxidation by-product, malondialdehyde (MDA), (Muniz et al., 2007). Studies by Prakasam et al. (2001) and Singh et al. (2007) revealed a significant rise in plasma MDA levels in exposed farmers than in controls. There is increasing evidence that OP and carbamate

induce oxidative stress through the generation of free oxygen radicals, leading to lipid peroxidation and DNA damage (Hazarika et al., 2003; Abdollahi et al., 2004; Vidyasagar et al., 2004; Shadnia et al., 2005). Muniz et al. (2007) reported MDA levels 4.9 times and 24 times higher in farm workers and applicators respectively than in controls. Pesticides induce a wide array of human health effects through oxidative stress causing cytogenetic damage and carcinogenicity (Mansour, 2004).

### **Chlopyrifos and oxidative stress**

Though accumulation of acetylcholine is said to be its mechanism of action, other putative mechanisms, including oxidative stress, have been implicated in molecular mechanisms of CPF toxicity (Ambali et al., 2010). CPF intoxication causes a significant decrease in reduced glutathione, catalase and glutathione-S-transferase activities (Goel et al., 2005). CPF-induced toxicity in the central nervous system may be due in part to the induction of oxidative stress (Slotkin 2004; Yu et al., 2008). CPF induces immune alterations, associated with lymphocyte subpopulations in rats (Blakley et al. 1999). It evokes oxidative stress, thereby interfering with signalling cascades and transcriptional events, involved in neural cell differentiation (Qiao et al., 2001).

### **Cypermethrin and oxidative stress**

Cypermethrin and other pyrethroids are metabolized in the liver via hydrolytic ester cleavage and oxidative pathways by the cytochrome P-450 enzymes to yield reactive oxygen species responsible for oxidative stress in mammals (Floodstrom et al., 1988; Klimek, 1990). Increased reactive oxygen species/free radical-mediated lipid peroxidation leads to cytotoxicity and genotoxicity in higher vertebrates during exposure (Kadous et al., 1994; Gassner et al., 1997; Kale et al., 1999). During metabolism of cypermethrin, it forms cyanohydrines, decomposing further to cyanides and aldehydes; substances that can induce production of reactive oxygen species (Wielgomas and Krechniack, 2007).

## **ANTIOXIDANTS**

An antioxidant is any substance that when present at low concentrations compared to that of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1999). The effect produced by reactive oxygen and nitrogen species is balanced by the antioxidant action of non-enzymatic antioxidants, as well as by antioxidant enzymes (Valko et

al., 2006). Such antioxidant defences are extremely important as they are involved in the direct scavenging of free radicals (prooxidants), thus providing maximal protection for biological sites. Research findings have established the use of many non enzymatic antioxidants in mitigating the adverse effect of these pesticides (Ambali et al., 2010; Ambali and Ayo, 2011; Kojo, 2004).

### **Vitamin C**

Vitamin C (ascorbic acid) is a very important and powerful antioxidant. Vitamin C functions together with the antioxidants. For example, it cooperates with vitamin E to regenerate  $\alpha$ -tocopherol from  $\alpha$ -tocopherol radicals in membranes and lipoproteins (Kojo, 2004). Vitamin C protects membranes against oxidative stress (Kojo, 2004). Ambali et al. (2010) and Ambali and Ayo (2011) showed that vitamin C ameliorates sensorimotor and cognitive changes induced by acute Chlopyrifos exposure in Wistar rats.

### **Vitamin E**

Vitamin E is a fat-soluble vitamin that exists in eight different forms.  $\alpha$ -Tocopherol is the most active form of vitamin E in humans. It is a powerful biological antioxidant, which is considered to be the major membrane-bound antioxidant employed by the cell (Pryor, 2000). Its main antioxidant function is the protection of cytomembranes against lipid peroxidation (Valko et al., 2006). Ambali and Aliyu (2012) showed that vitamin E mitigated the short – term sensorimotor and cognitive changes induced by acute chlopyrifos exposure in Wistar rats, due to its antioxidant and acetylcholinesterase restoration properties.

### **Flavonoids**

They are polyphenolic compounds which constitute one of the most commonly occurring and ubiquitous groups of plant metabolites, and they represent an integral part of human diet (Rice-Evans, 2001). One of the most actively studied properties of flavonoids is their protection against oxidative stress (Polovka et al., 2003). Flavonoids are ideal scavengers of peroxy radicals due to their favourable reduction potentials relative to alkyl peroxy radicals. They are thus, in principle, effective inhibitors of lipid peroxidation (Polovka et al., 2003).

### **Melatonin**

Melatonin is a free-radical scavenger and a strong antioxidant, secreted by the pineal gland. The function of

this indole amine as antioxidant and free radical scavenger, is facilitated by the ease with which it crosses morphophysiological barriers for example blood-brain barrier, intracellular and subcellular barriers (Reiter et al., 1999).

Melatonin stimulates several important antioxidative enzymes, including superoxide dismutase (Albarran et al., 2001), glutathione peroxidase (Wakatsuki et al., 2001) and glutathione reductase (Pablos et al., 1997). Several studies suggest that melatonin reduces lipid peroxidation, which is an indicator of oxidative stress in the rat brain (Pekarkova, 2001; Ortega-Gutierrez et al., 2002). Melatonin also neutralizes the oxidative and inflammation process caused by ageing (Rodriguez et al., 2007). It has been documented that melatonin improved cognitive decline caused by free-radical generation in the brain of rats (Sharma and Gupta, 2001). Gonenc et al. (2004) also reported that melatonin exerts a positive effect on the Morris water maze performances which is a model used to evaluate the cognitive functions of rats. Umosen et al. (2012) showed that Melatonin ameliorated the adverse effects caused by subacute CPF exposure in pituitary gland and testis due to its antioxidant property.

It is necessary to find solutions to the problems presented by using pesticide formulations with cocktails of chemicals in them because of the adverse effects they possess despite their benefits. It is therefore, imperative to adopt measures that will ameliorate the toxic effects caused by the frequent, unavoidable exposure to these pesticides using antioxidants.

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