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## Dermal Exposure to Sub-Toxic Amount of Chlorpyrifos - Is It Neurotoxic?

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### 1. Introduction

Chlorpyrifos (O, O-diethyl-3, 5, 6-trichloro-2-pyridyl phosphorothioate) is an organophosphate (OP) pesticide widely used across the globe for the last 4 decades. It was registered in the United States as early as 1965. Although chlorpyrifos (CPF) has been principally used as pesticide in agriculture sector, its domestic use is found to be extensive in home-gardens as well as indoors to get rid of cockroaches, fleas, spiders and flies (Lemus & Abdelghani, 2000). It is also smeared on the body surface of the sheep and horse to eradicate lice and fleas as well as for the treatment of dog kennels. Farmers are exposed to CPF and other OP pesticides over their skin by direct contact as well as by inhalation during preparation of the spray solutions, loading of spray tanks and application of the pesticides. Acute exposure to CPF by dermal, oral and inhalation route was moderately toxic and US Environmental Protection Agency categorized it as a class II toxin (Eisler, 2000). All OP insecticides act by inhibiting the enzyme acetylcholinesterase (AChE), and thereby increase the levels of acetylcholine in the synapses. Excessive stimulation of the cholinergic post-synaptic receptors leads to cholinergic toxicity. Acute poisoning produced by accidental ingestion or inhalation of OP pesticides like chlorpyrifos causes non-lethal symptoms like nausea, vomiting, abdominal cramps, diarrhoea, excessive salivation and headache. Such poisoning may also give rise to blurred vision, muscle twitches, difficulty in breathing, random jerky movements and convulsion. Symptoms usually occur within hours of exposure and with new AChE being synthesized, after few weeks the symptoms of cholinergic toxicity disappear.

Apart from the acute cholinergic toxicity affecting the central nervous system, organophosphate pesticides also affect specific areas of the brain. These areas include the parts of the cerebral cortex which is responsible for cognition and short term-memory. Three well-designed epidemiological studies examined the patients previously poisoned by OP pesticides several years after hospitalization and found deficits in cognitive tests without any neurological abnormality. One study included 100 patients admitted to the hospital and followed nine years after the poisoning. Comparison was done with matched controls (Savage et al., 1988). Significant deficit in several cognitive tests of memory and abstraction was found among the pesticide affected patients. But neurological physical examination and electroencephalographic examination were inconclusive. A second study (Rosenstock et al., 1991 and McConnell et al., 1994) involved 36 men poisoned by OP pesticides (mainly methamidaphos). They were followed two years after hospital admission. Cognitive deficits

were observed in poisoned patients compared to the matched controls. These patients also showed significant decrease in vibrotactile sensitivity which was presumed to be an indicator of peripheral neuropathy. The third study (Steenland et al., 1994) also found deficit in sustained attention among OP pesticide affected people 7 years after the poisoning. This study involved 128 people poisoned with OP pesticides. OP pesticide induced neurotoxicity in the humans and other animals has been proposed to occur via three distinct actions: cholinergic neurotoxicity, organophosphorus ester-induced delayed neurotoxicity (OPIDN), and organophosphorus ester-induced chronic neurotoxicity (OPICN) (Abou-Donia, 2003).

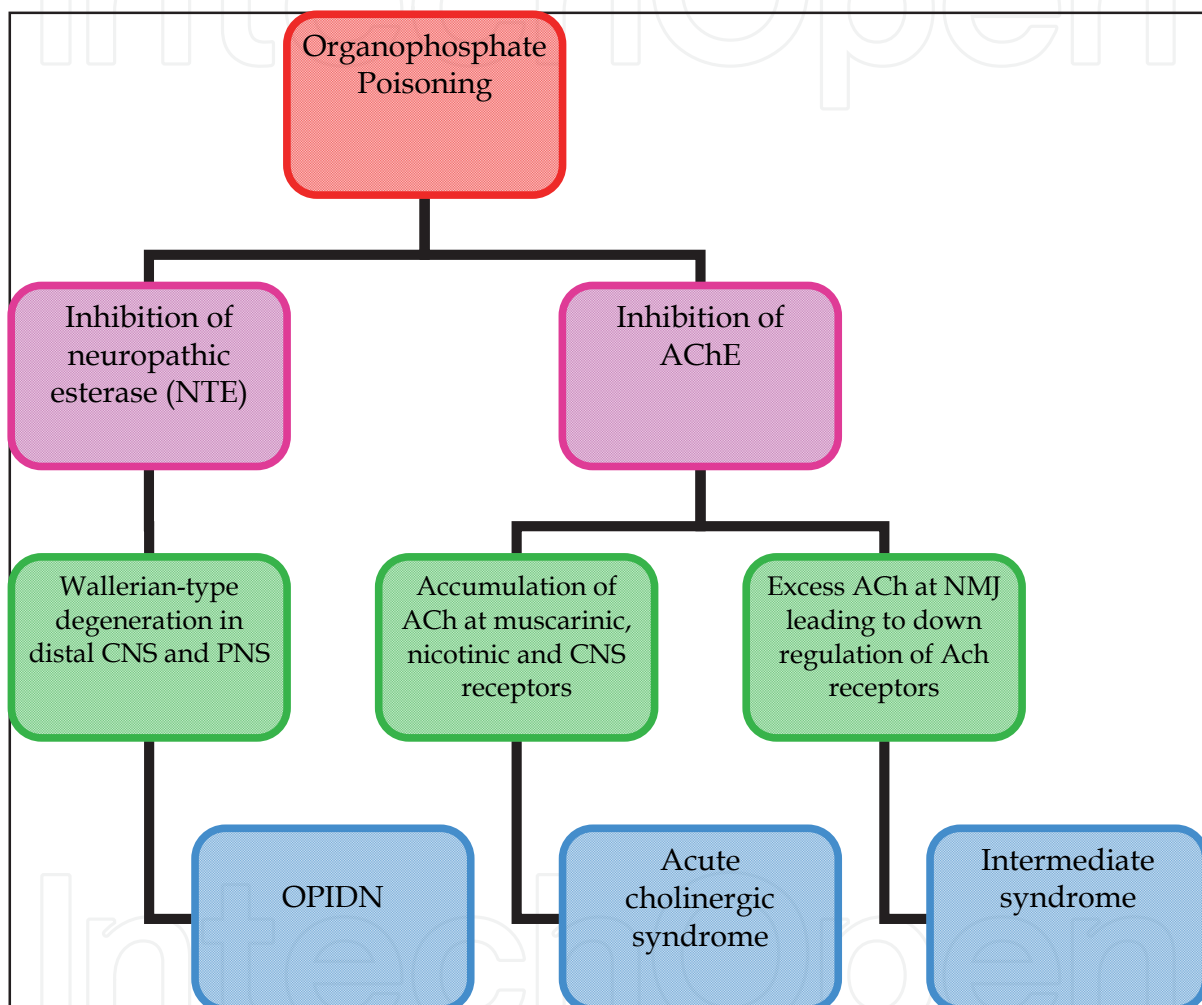


Fig. 1. Classification of organophosphate poisoning. ACh = Acetylcholine; CNS = Central nervous system; NMJ = Neuromuscular junction; OPIDN = Organophosphate-induced delayed neuropathy; PNS = Peripheral nervous system. Source: Jones & Karalliedde 2006, Davidson's Principles and Practice of Medicine, 20<sup>th</sup> edition

Although adverse effects of ingestion of CPF in sub-toxic doses by oral and inhalation methods have been proven by many studies, the general perception prevails that dermal exposure to chlorpyrifos is not as significant or as dangerous as other routes of exposure. Hence dermal exposure has not been given enough attention by the farmers and pesticide industry workers particularly in developing countries. CPF is absorbed through the skin and absorption through the skin may result in systemic intoxication. CPF and its metabolites

have been suggested to establish a reservoir and accumulate in skin resulting in longer exposure duration and more adverse long-term effects. The intensity of absorption of CPF through the skin depends on the solvent used and is usually slower than the uptake by other routes. Single dermal application of CPF diluted in ethanol for 4 hours on human volunteers was found to cause absorption of 4.3% of the applied dose and CPF was retained by the skin with mean elimination half-life of 41 hrs (Meuling et al., 2004). Application of a sub-clinical single dermal dose of CPF, 30 mg/kg, on pregnant Sprague-Dawley rats inhibited maternal and foetal brain AChE activity within 24 hours of exposure (Abu-Qare et al., 2001). The dose of a toxic material that causes death in one-half of the test population, when it is given on a short-term basis is described as its lethal dose (LD50). An acute dermal toxicity study on rats using chlorpyrifos soluble in xylene found that acute dermal LD50 for male rats was 202 mg/kg (Gaines, 1969). CPF was considered moderately toxic by oral route with an oral LD<sub>50</sub> of 223 mg/kg in rats. The acceptable daily intake (ADI) for CPF by oral route as pesticide residues in food was found to be 0.003 mg/kg/body weight (Barden, 2011, ADI list, Australia). ADI is the level of intake that can be ingested daily over the life time with no appreciable health risk. Cholinesterase activity in RBC and serum has been used as a method of surveillance or biological monitoring of exposure to OP pesticides, particularly for screening of workers exposed to OP pesticides. Dermal exposure to 10 mg/kg/day of CPF in rats was found to cause RBC cholinesterase inhibition of by 16% after 4 days of application. NOEL (No observed adverse effect level) dose of dermal CPF exposure was found to be 5mg/kg/day (Donovan, 2006). The neurobehavioural, neurochemical and neurohistological studies have been done using the animal models of dermal exposure to the mixtures of OP pesticides (Abdel-Rahman et al., 2001; Abdel-Rahman et al., 2004; Abou-Donia et al., 2004). However, the morphological effect of dermal exposure to sub-toxic dose of only chlorpyrifos, the widely used pesticide in the developing world, on the central nervous system has been studied by Lim KL, Tay A, Nadarajah VD and Mitra NK (2011). Although Mitra NK et al., (2008) and Mitra NK et al., (2009) studied the neurotoxic effect of dermal application of low dose chlorpyrifos in the hippocampus and neurotoxic effect of concurrent application of stress and dermal application of low dose chlorpyrifos in the hippocampus, the doses used were 1/5<sup>th</sup> dermal LD50 and 1/2 dermal LD50 of chlorpyrifos. Lim et al., (2011) had used 1/5<sup>th</sup> dermal LD50 and 1/10<sup>th</sup> dermal LD50 of chlorpyrifos. The methodology, results and discussion of the study by Lim et al., (2011) have been incorporated in this chapter to explain the neurotoxic effect of dermal application of sub-toxic doses of chlorpyrifos.

## 2. Materials and methods

### 2.1 Dermal application of CPF and estimation of Cholinesterase (AChE)

Commercial preparations of CPF (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate) manufactured in Kuala Lumpur, Malaysia was used in this study. This preparation contained 38.7% W/W CPF diluted in xylene. Male Swiss albino mice (species: ICR), 60 days old (30-32 g) were used in this study. They were housed in plastic cages (six in a cage) and were exposed to natural, twelve hourly light and dark sequence. Lab chow (pellet feed) and water were given ad libitum. Animal experiments adhered to the principles stated in the guide-book of laboratory animal care and user committee of the International

Medical University and in accordance with the declaration of Helsinki. The mice were divided into 3 groups ( $n = 6$ ). The experiment was conducted in two phases (one with period of experiment for 7 days and another with period of experiment for 3 weeks).

Application of CPF on the tail skin of albino mice was done in the dose regimen of  $1/5^{\text{th}}$  dermal LD50 and  $1/10^{\text{th}}$  dermal LD50 for 7 days and 3 weeks (Fig.2). Surgical gauze smeared with the CPF in xylene solution (1 ml) was wrapped around the tails and a barrier of aluminium foil was applied to prevent the solution from evaporation. Daily exposure was maintained for 6 hours which was similar to the daily dermal exposure time in the agricultural workers to the pesticides.

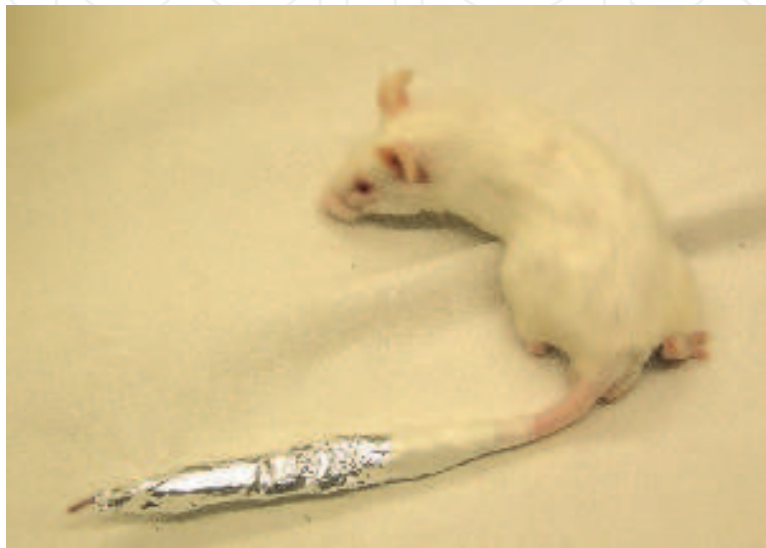


Fig. 2. Dermal application of CPF on the tail of the mouse under occlusive bandage.

A control group was maintained with exposure to dermal application of 1 ml of xylene solvent for similar duration. Amplex Red acetylcholinesterase assay kit, an ultrasensitive method for monitoring serum AChE concentration in a fluorescence microplate reader was used. The serum samples were collected at the end of 7 days and 3 weeks in two phases of the experiment. The mean serum AChE expressed as U/ml was subjected to one way ANOVA statistical analysis followed by Post hoc LSD test.

## 2.2 Qualitative and quantitative studies of neurons and the glial cells in the hippocampus

The sample of forebrains collected from the groups of treated mice at the end of 7<sup>th</sup> day (phase I) and at the end of 21<sup>st</sup> day (phase II) were fixed in 10% formal saline. Hippocampal area was trimmed off by making coronal section between the optic chiasma and the infundibulum. The portion of the brain was then divided into left and right lobes by a single saggital slice. This allowed the same mouse brain to be stained by two different stains. The brain tissues were processed and embedded in paraffin. The left half sections (8 micron) were stained with 0.2% thionin (Nissl stain) and used for qualitative and quantitative histomorphometric study of hippocampal neurons. Right half sections (4 micron) were used for immunohistochemical stains for Glial Fibrillary Acidic Protein (GFAP). For Nissl stain, every subsequent 10<sup>th</sup> section was collected. To obtain similar sections in the right lobe, every subsequent 27<sup>th</sup> section was collected. Every 10<sup>th</sup> paraffin section (5 slides in each

animal) stained with 0.2% thionin, containing hippocampal area, was chosen from each animal in groups of treated mice and a quantitative study of the normal looking neurons was done. The slides were examined and photographed under 400X magnification with the help of a photographic camera attached to the microscope. Selection of the hippocampal area for neuronal count was done by randomly choosing two areas of CA1, one area of CA2 and two areas of CA3 parts of the hippocampus observed in a section. Image-Pro Express software was used to count neurons with prominent nucleolus within a measured rectangular area in the selected regions. Random measurements of neuronal cell diameter were also taken for each region. The absolute neuronal density (P) per unit area of section was estimated using the formula  $P=A \times M / L+M$  (Aberchrombie 1946); M= Section thickness in micron (8 micron); L = Mean nuclear diameter of respective area; A = Crude neuronal count per sq.mm of section. The astrocytes with processes were stained brown with the immunohistochemical stain for GFAP filaments, particularly in stratum lacunosum-moleculare of the mouse hippocampus. The numbers of astrocytes with prominent processes were counted within a measured rectangular area. Three such areas were randomly selected in the every 27<sup>th</sup> section (3 sections in each animal). Both the mean neuronal density quantified under Nissl stain and mean astrocytic density quantified under GFAP immuno-stain expressed as values per sq mm of section, were subjected to One way ANOVA statistical analysis followed by Post hoc Bonferroni test to find out inter-group difference.

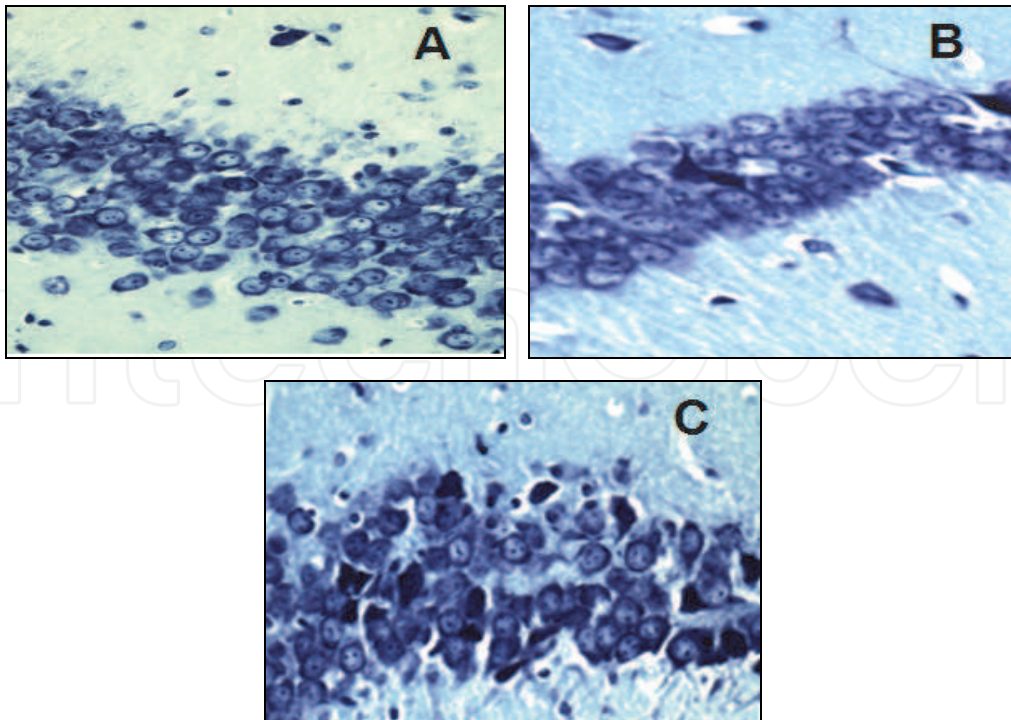
### 3. Results

#### 3.1 Changes in serum cholinesterase following dermal application of CPF

Depletion of serum cholinesterase concentration in the mice group exposed to 1/5<sup>th</sup> LD50 of CPF for 3 weeks was 95.9% compared to the mean AChE level in the control group. The change was statistically significant ( $p<0.05$ , One way ANOVA, Post hoc LSD). On the other hand, dermal application of 1/5<sup>th</sup> LD50 of CPF for 1 week caused depletion of serum AChE by 80.2%. The change was also statistically significant ( $p<0.05$ ). Dermal exposure to 1/10<sup>th</sup> LD50 of CPF for 3 weeks caused depletion of serum AChE by 88.3% compared to the control. The change was statistically significant ( $p<0.05$ ). However when 1/10<sup>th</sup> LD50 of CPF was dermally applied for only 7 days, depletion of serum AChE was 30.5% and was not significant statistically.

#### 3.2 Changes in the neuronal density of hippocampus following dermal application of CPF

The mean neuronal density per sq.mm of the section in histomorphometric study of the hippocampus was reduced by 24.7%, 18.4% and 22% compared to the control in CA1, CA2 and CA3 hippocampal areas in the group of mice exposed to 1/5<sup>th</sup> LD50 of CPF for 3 weeks. All the changes in the three areas were statistically significant ( $p<0.001$ , One way ANOVA, Post hoc Bonferroni). When the application was done for only 7 days, the reduction in mean neuronal density was 15.3%, 26% and 27% respectively in CA1, CA2 and CA3 hippocampal areas. The changes were statistically significant compared to the control ( $p<0.05$ ) in CA1 and CA2 hippocampal areas. The reduction was most significant ( $p<0.001$ ) in CA3 hippocampal area. Hence even with 7 days dermal exposure to 1/5<sup>th</sup> LD50 of CPF, the neurotoxicity in the hippocampal area was significant.



- A. Control group showing prominent perinuclear nissl granules  
 B. Group applied with 1/10th dermal LD50 of CPF for 7 days, showing no apparent damage except few pyknotic neurons (dark coloured)  
 C. Group applied with 1/5th dermal LD50 of CPF for 7 days, showing many pyknotic neurons (dark coloured) [Nissl stain with Thionin, 400x, 8 $\mu$ ]

Fig. 3. Photomicrograph of hippocampal CA3 neurons in different groups of mice

Exposure to 1/10<sup>th</sup> LD50 of CPF for 7 days was however least toxic. It reduced the mean neuronal density by 7.6%, 13.6% and 21% in CA1, CA2 and CA3 hippocampal areas compared to the control. The change in CA3 area only was statistically significant ( $p < 0.05$ ). The observation indicated that CA3 area of the hippocampus was more susceptible to neuronal damage following dermal exposure to low dose of CPF for only 7 days (Fig. 3). Even when applied dermally for 3 weeks, the dose of 1/10 LD50 of CPF was found to be less neurotoxic. The mean neuronal density was reduced by 9%, 11% and 9.6% in CA1, CA2 and CA3 hippocampal areas in the group receiving dermal application of 1/10 LD50 of CPF for 3 weeks. One way ANOVA did not show any significant difference in the mean neuronal density in the three areas in this experimental group.

### 3.3 Changes in the astrocytic density in the hippocampus following dermal application of CPF

Examination of the photomicrographs revealed that following one week of application, longer and more numerous astrocytic processes were observed in the group exposed to 1/5<sup>th</sup> LD50 of CPF compared to the group exposed to 1/10<sup>th</sup> LD50 of CPF (Fig. 4) in stratum lacunosum-moleculare and stratum oriens of the hippocampus. Quantitative study showed that the mean astrocytic density per sq. mm of the section was raised in all groups receiving dermal applications of CPF for 7 days. An increase of 37.2% in mean astrocytic density was observed in the group exposed to 1/10<sup>th</sup> LD50 of CPF compared to the control, while an increase of 41% was seen in the group exposed to 1/5 LD50 of CPF. Both the changes in the

1/10<sup>th</sup> LD50 of CPF group and the 1/5 LD50 of CPF group were statistically significant ( $p < 0.001$ , One way ANOVA, Post hoc Bonferroni). Compared to the application of CPF for 7 days, application for 3 weeks did not produce prominent visible changes in the expression of GFAP. The mean astrocytic density was increased by 9% in the mice group receiving dermal application of 1/10<sup>th</sup> LD50 of CPF for 3 weeks compared to the control. In the group receiving dermal application of 1/5<sup>th</sup> LD50 of CPF, the density of astrocytes was raised by 9.5%. One way ANOVA test did not show any significant inter-group difference in the mean astrocytic density between the control group, 1/10<sup>th</sup> LD50 of CPF group and 1/5<sup>th</sup> LD50 of CPF group in the phase II experiment (3 weeks).

7 days application	CA1	CA2	CA3
Control	881.8 (146)	710.5 (146)	640.7 (75)
CPF 1/10 LD50	814.7 (158)	613.8 (125)	504.3* (116)
CPF 1/5 LD50	768.7# (201)	578.7# (103)	483.3# (167)
3 weeks application			
Control	1098.3 (116)	642.7 (72)	639.1 (67)
CPF 1/10 LD50	998.4 (72)	571.8 (70)	577.7 (85)
CPF 1/5 LD50	826.8# (108)	524.1# (77)	496.9# (40)

Table 1. Mean (S.D) neuronal density per sq.mm of the section in different treatment groups in three hippocampal areas. # Significantly reduced in CPF 1/5 LD50 groups compared to the control group ( $p < 0.05$ , One way ANOVA Post hoc Bonferroni) ; \* Significantly reduced in CPF 1/10 LD50 groups compared to the control group ( $p < 0.05$ , One way ANOVA Post hoc Bonferroni).

	7 days application	3 weeks application
Control	256.9 (54)	317.4 (75)
CPF 1/10 LD50	352.6# (99)	347.2 (70)
CPF 1/5 LD50	362.5# (96)	347.6 (84)

Table 2. Mean (S.D) astrocytic density per sq.mm of the section in different treatment groups stratum lacunosum-moleculare of the hippocampus. # indicates significant increase compared to the control group ( $p < 0.05$ , One way ANOVA Post hoc Bonferroni).



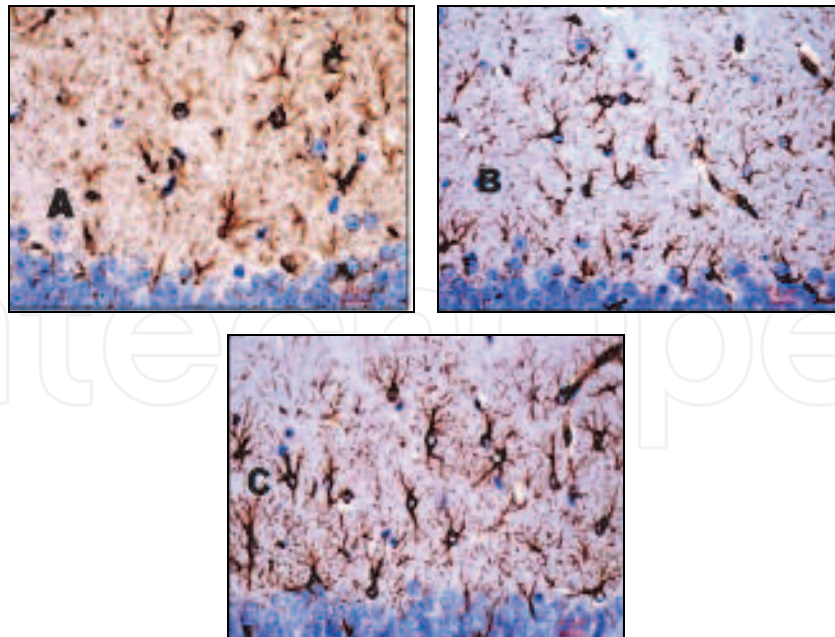


Fig. 4. Photomicrograph showing the immunohistochemical staining of GFAP expression in stratum moleculare-lacunosum of the hippocampus in groups of mice at the end of 7 days of experiment. Brown colour rounded cells with processes are the astrocytes. A-Control group; B- 1/10<sup>th</sup> LD50 of CPF group; C- 1/5<sup>th</sup> LD50 of CPF group (400X, GFAP stain, 4 $\mu$ )

#### 4. Discussion

Somewhat similar to this study, Latuszyńska et al.,(2001) used dermal application of 1/70 dermal LD50 of CPF along with 0.5 mg/ cm<sup>2</sup> surface area of cypermethrin and plasma cholinesterase was reduced by 81%. The reduction of plasma cholinesterase was 92% when 1/14<sup>th</sup> dermal LD50 of CPF in combination with cypermethrin 2.7 mg/cm<sup>2</sup> was applied dermally for 1 week. This study found depletion of serum AChE by 30.5% only when 1/10 dermal LD50 of CPF was applied for 7 days. Serum AChE enzyme is produced in the liver and it is a reliable measure for detecting acute OP toxicity. Among general population, approximately 2 to 3% has a genetic variation of serum cholinesterase deficiency. Acute or chronic inflammatory conditions, malnutrition, liver disease and physiological condition like pregnancy can produce AChE deficiency. The depletion of AChE in these conditions is not as severe as observed following exposure to OP pesticides. Measuring cholinesterase activity in RBC is used as a method of surveillance for detection of exposure to OP pesticides. This is applied mainly to the workers working in pesticide industry. The role of AChE is to terminate impulse transmission at the cholinergic synapses within the nervous system by hydrolyzing acetylcholine (ACh) into choline and acetate allowing recycling of hydrolysed substrates into new neurotransmitter (Rang et al., 2001). As a consequence of the inhibition of AChE, ACh accumulation occurs at the synapses. ACh is found throughout the CNS and it is present in relatively higher concentration in the cerebral cortex, thalamus and various nuclei of the basal forebrain. As a neuromodulator, ACh has multiple effects on the CNS. Through synaptic plasticity, it plays a prominent role in learning involving the neo-cortex and hippocampus. ACh has been found to enhance the amplitude of synaptic potentials following long term potentiation in dentate gyrus, CA1 hippocampus, pyriform

cortex and neocortex. It most likely acts either by suppressing or by enhancing the current via NMDA (N-methyl-D-aspartic acid) receptors (Yu and Dayan, 2005).

In the present study on dermal application of CPF, evidence of neuronal damage was found in the stratum pyramidale of the hippocampus. Qualitative observations of the hippocampal neurons in this study showed that following seven days of low dose CPF application (1/10th dermal LD50), no apparent damage to the neurons was visible. However at the higher dose (1/5th dermal LD50), seven days of application resulted in visible damage in the form of pyknosis. Dendritic morphology was assessed in the prefrontal cortex, CA1 area of the hippocampus and the nucleus accumbens following repeated (14 days) low dose intraperitoneal application of OP malathion (40 mg/kg BW) in mice. Dendritic length in the hippocampus and prefrontal cortex, and density of dendritic spines in all the three areas assessed were reduced (Campana et al., 2008). As part of the trisynaptic circuit, afferent inputs to the hippocampus are first sent to the dentate gyrus, which then projects to the CA3 area. The CA3 neurons then send projections to CA1. Dendrites of CA1 neurons project to the subiculum and then back to the entorhinal cortex. CA3 being an early structure in this circuit, it is the first part of the hippocampus to be affected by cholinergic overactivity. This could explain the significant ( $p < 0.05$ ) neuronal reduction observed only in CA3 hippocampal area after application of low dose CPF (1/10th dermal LD50) for seven days. This also indicated that CA3 area of the hippocampus was more susceptible to neuronal damage following dermal exposure to low dose of CPF. Agricultural workers chronically exposed to low-levels of CPF and other pesticides were found performing poorly on neurobehavioral tests (Rothlein et al., 2006). These functional deficits can be extrapolated to be caused by prolonged exposure to low dose CPF.

Following one week of CPF application at both doses (1/10th and 1/5th dermal LD50), GFAP expression as measured by astrocytic density was significantly increased compared to the control group. GFAP expression has been found to be increased following toxic insult to the CNS in many studies. A single subcutaneous injection (50  $\mu\text{g}/\text{kg}$  bw, 1/2 LD50) of the cholinesterase inhibitor Sarin was found to significantly increase GFAP levels in the cerebral cortex by 269% after one hour, and to 318% after two (Damodaran et al., 2002). Qualitative examination showed that following seven days of CPF application, GFAP expression in the astrocytes was more prominent compared to the control groups. The astrocytic processes of the groups receiving CPF were longer, and greater in number. This may be attributed to the neuroprotective effect of astrocytes limiting neuronal damage. It has been suggested that the metabolites of CPF, trichloropyridinol (TCP), exert strong toxic effects on astrocytes, compromising their neuroprotective effects and thus increasing the neurotoxicity of CPF (Zurich et al., 2004). The neuroprotective effects of astrocytes have been suggested in many studies. To assess the influence of glial cells on the neurotoxicity of OPs, aggregate brain cell cultures of foetal rat telencephalon were treated with CPF and parathion for 10 days. The study by Zurich et al., found that the neurotoxicity of CPF and parathion was increased in aggregate cultures deficient in glial cells.

This study observed both neuronal damage as well as GFAP expression following low dose dermal application of CPF. It was observed that with increasing neuronal damage, GFAP expression was more and the mean astrocytic density was increased. Exposure to 1/10<sup>th</sup> LD50 of CPF for 7 days reduced the mean neuronal density by 7.6%, 13.6% and 21% in CA1, CA2 and CA3 hippocampal areas. This group showed 37.2% increase in mean astrocytic density in stratum lacunosum-moleculare compared to the control group. In contrast, when

1/10<sup>th</sup> LD50 of CPF was applied for 21 days, a low level of neurotoxicity was produced reducing mean neuronal density by 9%, 11% and 9.6% in CA1, CA2 and CA3 hippocampal areas. This low level of neurotoxicity produced a moderate glial reaction as evidenced by 9% increase in astrocytic density compared to the control group which was not found to be statistically significant. The findings of this current study support previous suggestions that astrocytes provide neuroprotection. Although CA3 hippocampal area was found to be most susceptible out of the three main Cornu-Ammonis areas of the hippocampus towards the neurotoxic effect of low dose chlorpyrifos, the level of neurotoxicity was less (9.6% reduction in neuronal density) and insignificant when low dose (1/10 LD50) CPF was applied for 3 weeks. Comparatively when the similar dose was applied for 7 days a higher (21% reduction in neuronal density) and significant level of neurotoxicity was observed. As evidenced by the glial reaction, the level of neurotoxicity produced by application of low dose CPF for 3 weeks was less. Hence regeneration of the neurons was possible in the hippocampus Cornu-Ammonis pyramidal layer which might have been reflected in the lower levels of reduction in the neuronal density. Previous literature has suggested that neurogenesis is possible in hippocampus of adult rodents and human (Eriksson et al., 1998).

## 5. Conclusion

In conclusion, the study by Lim KL, Tay A, Nadarajah VD and Mitra NK (2011) has shown that the dermal application of chlorpyrifos, an organophosphate pesticide in the dose of 1/5<sup>th</sup> dermal LD50 was capable of producing significant neurotoxicity measured in the parameters of serum cholinesterase reduction, hippocampal neuronal density reduction as well as increased GFAP expression when applied for a short term period of 7 days or prolonged application period of 3 weeks. However a low dose dermal application of chlorpyrifos in the dose of 1/10<sup>th</sup> dermal LD50 produced a reduced level of neurotoxicity. Initial phase of neurotoxicity produced by a comparatively shorter duration of dermal application of low-dose chlorpyrifos stimulated significant glial reaction in the form of GFAP expression. The pesticide applicators should avoid exposure of chlorpyrifos containing pesticides to their skin to prevent neurotoxic effects of chlorpyrifos.

## 6. Acknowledgment

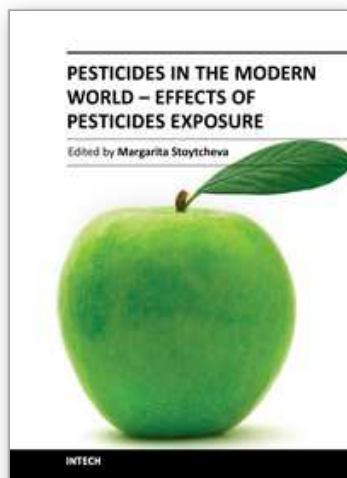
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## **Pesticides in the Modern World - Effects of Pesticides Exposure**

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The introduction of the synthetic organochlorine, organophosphate, carbamate and pyrethroid pesticides by 1950s marked the beginning of the modern pesticides era and a new stage in the agriculture development. Evolved from the chemicals designed originally as warfare agents, the synthetic pesticides demonstrated a high effectiveness in preventing, destroying or controlling any pest. Therefore, their application in the agriculture practices made it possible enhancing crops and livestock's yields and obtaining higher-quality products, to satisfy the food demand of the continuously rising world's population. Nevertheless, the increase of the pesticide use estimated to 2.5 million tons annually worldwide since 1950., created a number of public and environment concerns. This book, organized in two sections, addresses the various aspects of the pesticides exposure and the related health effects. It offers a large amount of practical information to the professionals interested in pesticides issues.

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