CHAPTER IV

DISCUSSION

The present study showed that multiple doses of dicrotophos caused a decrease in AChE activity in plasma, RBC and brain at 24 hr after last dicrotophos injection. It is noted that the decrease in activity in plasma was more prominent than in red blood cell. This indicates that, though may not be associated with symptoms and signs of OP toxicity (Wessels *et al*., 2003), plasma AChE level is useful in detecting acute exposure to OP. In addition to the smaller decrease in AChE activity in red blood cell, the recovery of red blood cell AChE activity was seemingly slower rate than that of the plasma AChE level. This result indicates that the red blood cell AChE level is accepted as an index of chronic exposure to OP (Paul, 1987; Pongrawewongsa and Ruangyuttikarn, 1999).

Regarding the brain level of AChE activity, it can be noted that the brain level of AChE was shown with no significant recovery throughout the 3-wk experimental period after completing the OP injection. The results reflect the facts that the brain is a vital organ with almost no regeneration after reaching full development. Though it is impossible to monitor the brain level of AChE activity in people exposed to OP, the results indicate that the brain AChE should be regarded as a more sensitive indicator of dicrotophos exposure than plasma and RBC. Comparing to this relatively slow rate of brain AChE recovery, the faster recovery of RBC AChE activity might be due to the high re-synthesis or turnover rate of RBC synthesis (about 0.8% per day). The

findings with slow rate of recovery of brain AChE activity agree with a previous study in which another OP (DFP) was injected to rats for 22 day. It was observed in that study that plasma AChE activity had returned to a stable 100% of the control level by 5 to 6 day after last DFP dose while the recovery of brain AChE activity after termination of DFP dosing was retarded. Brain AChE activity was only about 65% of control at 28 day after last dose of DFP (Gearhart *et al*., 1994). Additional data showing that AChE activity in brain tissue is the most susceptible indicator of adverse toxic effect of dicrotophos in animal studies also supports the assumption (Dicrotophos: Health-based Reassessment of Administrative Occupational Exposure Limits, 2003).

The present study showed that multiple doses of dicrotophos decreased nerve conduction velocity in sciatic nerve at 3 wk after the last dicrotophos injection probably indicating the development of a peripheral neuropathy. This finding corresponds with the previous studies, in which another OP (dichlorvos) was administered to rats for 8, or 12 wk in a 5 day per week schedule, that caused a decrease in conduction velocity of the peripheral nerve (Dési and Nagymajtényi, 1999), and the findings that exposure to chlorfenvinphos, DFP or dimethoate for 4 wk produced nerve conduction deficit in the peripheral nerve (Papp *et al*., 2004) also support our results. In humans, the field studies of farmers exposed to OP pesticides reported the increased incidence of nerve conduction deficit from 7% to 35% in the neuropathy group (Jamal *et al*., 2002).

This present study showed that multiple doses of dicrotophos decreased the minimum thickness of myelin sheath in sciatic nerve at 3 wk after last

dicrotophos injection. In addition, sciatic nerve of dicrotophos-treated groups was observed with axonal swelling, axoplasmic vacuoles, condense cytoplasm and disaggregating myelin sheath. These findings agree with previous studies in which another OP (TOTP and TOCP) were observed to cause degeneration of myelinated nerve fiber in medulla, spinal cord and peripheral nerve and of which were correlated with the inhibition of NTE activity in brain and spinal cord (Jortner *et al*., 2005; Padilla and Veronesi, 1985). NTE is the target site for OP that induces delayed neuropathy. NTE is a 150-kDa transmembrane protein that localized to the cytoplasmic face of the ER. It is anchored via its N-terminal transmembrane segment (Li *et al*., 2003). In yeast lacking YMLO59c (NTE homologue in yeast) was reported with absence of glycerophosphocholine (GroPCho) production. The results indicated that YMLO59c is the enzyme responsible for deacylating phosphatidylcholine (PtdCho) to GroPCho (Zaccheo *et al*., 2004). In eukaryotic cells, PtdCho is the major membrane lipid that regulated by balancing between its synthesis and degradation via deacylation to GroPCho. When NTE activity is inhibited, disruption of membrane phospholipid homeostasis will occur. Other study found that in late pupae Swiss cheese (SWS; NTE homologue in *Drosophila*) mutants, glial cells form multilayered wrappings around neurons and axons. In adult SWS mutants, neurons in brain were observed with apoptosis and extensive vacuolation (Kretzschmar *et al*., 1997). Since NTE associated with phospholipid homeostasis in the ER and membrane phospholipid metabolism in glial cell, thus the inhibition of NTE activity by OP may result in disruption of axonal transport through the ER.

The decrease in nerve conduction velocity observed in this study was, probably partly, due to the decrease in minimum thickness of myelin sheath combined with myelinated fiber degeneration in sciatic nerve. Since myelin coating acts as an insulator to prevent current leakage across the myelinated portion of the membrane, myelination increases the speed of conduction of action potentials. Thus demyelination that observed in this study may result in nerve conduction deficit.

In sciatic nerve, the number of myelinated axon was significantly decreased only at 3 wk after last dicrotophos injection, while the minimum diameters were likely increased but no statistical significance was observed. This result showed that multiple exposures to dicrotophos caused a decrease in number of myelinated axon in sciatic nerve. This might indicate the probable cause of muscle weakness in legs observed in patients poisoned with OP insecticides. There is a study showing the increased incidence of muscle weakness from 0% in the non-neuropathic group to 17% in the neuropathy group of farmers exposed to OPs (Jamal *et al*., 2002). Other findings, in which OP insecticide (Dipterex) caused distal weakness of the legs, foot drop, difficult gate and muscle hypotonia (Vasilescu *et al*., 1984), also support our explanations. In addition, all hens treated with 20 daily injections of DFP exhibited nerve degeneration in the brain, spinal cord and peripheral nerves with impaired walking behavior (Sprague *et al*., 1980). The results from these studies demonstrated that exposure to some OP might cause demyelination of the nerve fiber leading to muscle weakness in the legs. Regarding the unmyelinated axon, the relatively same number and same minimum diameter

after dicrotophos exposure in both dicrotophos and saline-treated groups may indicate either no effects of dicrotophos on unmyelinated type of fibers in sciatic nerve or insensitivity of the fiber to the drug.

It is accepted that the lesions of neuropathy in animals as observed in fasciculus gracilis at medulla and cervical spinal cord level were prominent. However, in the present study, at medullary level, the numbers of myelinated axon were significantly decreased since 24 hr after last dicrotophos injection. Comparatively, the minimum diameters were significantly increased since 1 wk, later period. At cervical spinal cord level, the numbers of myelinated axon were significantly decreased since 1 wk after last dicrotophos injection while the minimum diameters were significantly increased only at 3 wk after last dicrotophos injection. Therefore, our result shows that, regarding the myelinated fiber in fasciculus gracilis, medullary fiber might be the most sensitive group affected by OP exposure. In this study the myelinated axon and unmyelinated axon were counted in 50 μm^2 cross sectional area. Thus in the same area the decreased numbers of myelinated axon may reflect the increased diameters of the axon in fasciculus gracilis at medulla and cervical spinal cord level.

By transmission electron microscopy, the myelinated fiber degeneration was observed with swollen dystrophic axons. There were vacuoles and condense cytoplasm within the axon. These findings agree with the previous study in which the lesion of OPIDN in rats was observed with myelinated axonal swelling with pallor or hyperchromatic staining and swollen dystrophic axon at medulla and spinal cord level (Jortner *et al*., 2005). The

lesions were reported to be restricted to rostral levels of the fasciculus gracilis and most prominent in the medullary region. The ultrastructural finding of these lesions was axoplasmic vacuoles, usually membrane-bound with some finely granular or flocculent material. Mitochondria and other organelles sometimes were aggregated at the margins of the vacuoles and some swollen axons were dilated by masses of membranous and tubular material, and dense bodies (Carboni *et al*., 1992). Other types of axonal lesions were observed as intraaxonal and intramyelin vacuoles which compressed the axoplasm to one side of the axolemma (Padilla and Veronesi, 1985). The results from these studies support the results in the present study.

Myelinated fiber degeneration that observed in fasciculus gracilis at medulla and cervical spinal cord in this study may be associated with sensory abnormalities in pesticide applicators. Since myelinated axons in fasciculus gracilis are responsible for sensory information regarding vibration, propioception, stereognosis and two-point discrimination from the trunk and lower extremities, these sensory processes will be disturbed after fiber degeneration. A previous study showed that pesticide applicators exposed to guthion had significantly less sensitivity to vibration than control group (Stokes *et al*., 1995). Other study showing the association between the incidence of neuropathy and the abnormal large fiber function as assessed by vibration threshold (Jamal *et al*., 2002) also support the assumption.

The minimum thickness of myelin sheath in fasciculus gracilis at both medulla and cervical spinal cord level were significantly increased only at 3 wk after last dicrotophos injection. The results indicate that prolonged

dicrotophos exposure caused an increase of thickness of myelin sheath. However, this finding was not comparable to the results from the previous study in which another OP (mipafox) was observed to cause swollen axons with thin myelin sheaths (Carboni *et al*., 1992). The increase in thickness of myelin sheath in the fasciculus gracilis at medulla and cervical spinal cord level following multiple exposures to dicrotophos was observed in this experiment while, by transmission electron microscopy, medulla and cervical spinal cord damages were observed with myelin debris, intramyelin vacuoles and disaggregating myelin sheaths. It is possible that the vacuoles, myelin debris, and disaggregating myelin sheaths, by itself, may cause the increase in thickness of myelin sheath. Supporting this assumption is the findings that exposure to TOCP produced spinal cord damage consisted of giant myelinated and demyelinated axonal swellings, myelin debris and vacuolated myelin sheaths (Padilla and Veronesi, 1985).

Regarding the unmyelinated axons in fasciculus gracilis at medullary level, the numbers of unmyelinated axon were significantly increased at 2 and 3 wk after last dicrotophos injection when compared with saline groups, while the minimum diameters were not different in any groups of animals. At cervical spinal cord level, the numbers of unmyelinated axon were significantly increased at 1, 2, and 3 wk after last dicrotophos injection when compared with saline groups, while the minimum diameters were not different in any groups of animals. The results indicate that prolonged dicrotophos affecting the numbers of unmyelinated axon in the fasciculus gracilis at medulla and cervical spinal cord level were most prominent at 2-3 wk period

after the exposure. This might indicate the probable cause of sensory abnormality observed in people poisoned with OP insecticides. There is a previous study in which 58% of 34 OP manufacturing workers were reported to have peripheral neuropathy including burning sensation in the palms and feet, numbness, reduced sensitivity and poor sensory localization (Ernest *et al*., 1995). Other study showed that the incidence of abnormal small fiber function, assessed by hot or cold sensation threshold from the dorsum of the foot, increased from 0% to 91% in the neuropathy group of people exposed to OPs (Jamal *et al*., 2002). Although the sensory pathway transmitting temperature sensation from the spinal cord to the thalamus is mediated via the spinothalamic tract, sensory abnormality that observed in people poisoned OP may be reflected unmyelinated nerve dysfunction.

In conclusion, prolonged exposure to dicrotophos affected AChE activity in RBC, plasma and brain, axon in sciatic nerve and fasciculus gracilis at medulla and cervical spinal cord level and nerve conduction velocity in sciatic nerve in rats. The decrease in nerve conduction velocity was probably due to the decrease in thickness of myelin sheath combined with fiber degeneration in the sciatic nerve. The findings indicate the probable prominent damages to myelinated axons and might be useful in describing muscle weakness caused by organophosphates. Also, myelinated and unmyelinated fiber degeneration observed in the fasciculus gracilis at medulla and cervical spinal cord level might be useful in describing locomotor and sensory abnormalities observed in persons exposed to the insecticides.