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Original article

Contractile property of skeletal muscle after dicrotophos exposure in rats

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Background: Dicrotophos is an organophosphate pesticide whose residue has been detected in most vegetables even in organic samples. Though this pesticide is classified as a highly hazardous Ib organophosphate, it is not banned in many countries including Thailand. Improper use of this chemical exposes the people to it through consumption of contaminated food and water. Some people develop signs of cholinergic syndrome following exposure and some suffer for days from paralysis of respiratory and limb muscles. However, there is no direct evidence indicating muscle weakness through decreased contractile property. All studies in humans thus far were clinically investigated and reported using information from subjective verbal histories.
Objective: To investigate the contractile characteristics of skeletal muscles after dicrotophos exposure.
Methods: A preliminary study was performed to determine the effective dose of dicrotophos that causes at least 30 % reduction in red blood cell cholinesterase activity. The rats were injected with dicrotophos daily at $LD_{6.25}$, $LD_{12.5}$ and LD_{25} doses for 5 weeks. It was found that the $LD_{12.5}$ dose caused the effect, starting at the 4th week of injection. Therefore, this dose was injected into the rats before examining the isometric twitch characteristics of gastrocnemius muscle and cholinesterase activity in red blood cells and muscle homogenates.
Results: Significant decreases in peak tension and time to peak tension were observed in rats exposed to this dose of dicrotophos. These decreases agreed with cholinesterase activity in RBC and muscle homogenates.
Conclusion: Dicrotophos exposure in rats caused decreased contractile activity providing direct evidence implying the muscle weakness often found in humans.

Keywords: Acetylcholinesterase (AChE), contractile property, dicrotophos, organophosphate, rat, skeletal muscle.

At present, new technologies for enhancing food production, including the use of insecticides in agriculture, have caused numerous health and environmental problems. This is partly due to inappropriate use of insecticides. It has been established that most of the people who suffer from exposure to the organophosphate (OP) group do so through consumption of contaminated water and vegetables. Workers can be exposed to OP while handling, mixing, or applying the chemicals.

Patients exposed to OP insecticides may show observable signs of cholinergic syndrome 2-24 hrs after the exposure [1]. After that, some may show signs of intermediate syndrome within 1-4 days [2] with paralysis of respiratory and proximal limb muscles for days. A rather systematic approach to investigating the relationships of the intermediate syndrome to cholinesterase inhibition and electromyographic findings was performed, but consistent positive relationships were not suggested, even though the syndrome was expected to result from combined pre- and post-synaptic dysfunction of neuromuscular transmission. Biopsy results could not explain the severe muscle weakness observed in the syndrome [3].

Chronic effects of OP exposure have not been studied systematically. All studies reported so far were obtained from subjective verbal histories, so the results are not conclusive [4]. In contrast, the effects of OP exposure in animals have been investigated in several species, including rats [5-8].

Disturbances in neural function were observed in studies showing the effects of OP on the electrical activity of muscles [9, 10]. In addition, OP was observed to affect slow twitch muscles more than fast twitch muscles [11]. It was noted that slow twitch muscle fibers from the soleus muscle were more

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sensitive to OP than fast twitch fibers from the extensor digitorum longus muscle. OP was also shown to affect the muscles, suggesting that an increased acetylcholinesterase (ACh) concentration at the motor end plate could damage the muscle fibers [12]. Exposure to OP caused persistent desensitization of the post-synaptic nicotinic receptor, leading to muscle weakness [13]. Recently, one OP, metylparathion, was studied at Chiang Mai University. It was injected into rats for 4 weeks and decreased red blood cell levels of Acetylcholinesterase (AChE) activity correlated with AChE activity sustained at the motor end plate [14].

The purpose of the present study was to study effects of dicrotophos, an OP that acts as a systemic pesticide, on contractile activity of skeletal muscles in rats. The present study was also aimed at emphasizing the hazardous effects of dicrotophos since this chemical is not banned in many countries, including Thailand, but is detectable in most fresh vegetable samples, even in those that are allegedly chemical-free [15, 16]. It was hypothesized that dicrotophos can be shown to directly, not just clinically, affect contractile activity of skeletal muscle. The findings might be useful in explaining clinically observed muscle weakness in patients who have been exposed to the organophosphate.

Materials and methods

Chemicals

The dicrotophos used in the present study was Canose 33® [$C_8H_{16}NO_5P$, 33% W/V SL dimethyl (E)-1-methyl-2-(dimethylcarbamoyl)-vinylphosphate] purchased from Superior Chemical Industry (Thailand) Ltd. The dicrotophos was diluted in 0.9% saline for intraperitoneal injection.

Animals

Male Wistar rats (200-250 g) were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. They were housed individually in controlled environmental conditions of 25 $\pm$ 1°C temperature, 50 $\pm$10 % humidity and 12/12 hrs lighting cycle. Water and food were given ad libitum. Body weight and food intake were checked throughout the experiment. The experimental protocol, including the preliminary study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University, Thailand.

Preliminary study: Selection of the effective dose of dicrotophos

The animals were divided into three groups and were intraperitoneally injected with dicrotophos daily at $LD_{6.25}$, $LD_{12..5}$ and LD_{25} doses [17] for 5 weeks. AChE activity was examined in blood collected from tails once a week. This part of the experiment was done in order to determine the effective dose that causes at least 30% reduction in red blood cell AChE activity. This dose was used in the remainder of the experiment.

Treatment of animals with the effective dose of dicrotophos

The daily dose and duration of dicrotophos ($LD_{12.5}$, 3.75 mg/kg BW for 4 weeks) were chosen for further study based on the preliminary results. Saline injection was given as a control. After the injection protocol was complete, the animals were allowed to rest for 24 hours before the contractile property of the gastrocnemius muscle was studied. The AChE activity in red blood cell and gastrocnemius muscle homogenate was determined thereafter.

Study of the contractile property of gastrocnemius muscle

The animals were anesthetized with pentobarbital sodium, 30 mg/kg body weight, by intraperitoneal injection. One lower limb of the animal was immobilized and the sciatic nerve was carefully exposed and severed. The distal nerve was placed on a stainless steel bipolar stimulating electrode connected to an electronic stimulator. The Achilles tendon of the gastrocnemius muscle was isolated, detached from its insertion and then tied and connected to a force-displacement transducer for monitoring its contractile property on a PowerLab computer via a bridge amplifier.

After completing the tendon and nerve preparation, the animal was left to rest for at least 30 minutes prior to the study of the contractile property of the underlying gastrocnemius muscle. The exposed sciatic nerve was thereafter stimulated with a single square wave pulse of 0.2 ms duration (Grass Instruments S48 Stimulator). The intensity of current was initially 5 volts increased in 1-volt steps until the maximal response was obtained. The muscle length was then adjusted to obtain its optimal length. The isometric contraction was initiated by a single

stimulation at a supramaximal voltage and the myogram of the muscle twitch was recorded using Chart program set at 100 mm/sec sampling rate. The peak twitch tension (PT), time to peak tension (TPT) and one-half relaxation time ($RT_{1/2}$) were determined from the myogram using the cursor marker (**Fig. 1**). The PT amplitude and TPT duration were defined from the point at which the tension rose from the basal line to the point whose tension was highest. The $RT_{1/2}$ was defined as the duration from the point that the PT was obtained to the point that the muscle relaxed to a half of its tension.

At the end of the experiment, a blood sample was collected and the gastrocnemius muscle was immediately excised, blotted dry, weighed with an electronic balance and kept frozen in liquid nitrogen for further determination of AChE activity.

Determination of AChE activity by biochemical assay

The AChE activity in red blood cells and muscle homogenates was determined following the method described by Ellman et al [18]. Briefly, enzyme activity was determined by measuring the yellow color produced when thiocholine reacts with dithiobisnitrobenzoate ion, and absorbance of the solution was measured at 405 nm using a spectrophotometer. AChE activity levels in red blood cells were calculated and reported in U/L while those in muscle homogenates were calculated and reported in U/L/g tissue.

Statistical analysis

All values of AChE activity were expressed as means $\pm$ SE. The significance of differences among experimental groups was determined using the Mann-Whitney U-test. A level of $p < 0.05$ was considered as a significant difference.

Results

Preliminary study

After daily injection of 3 different doses of dicrotophos ($LD_{6.25}$, $LD_{12.5}$ and LD_{25}) into rats for 5 weeks, all animals receiving the LD_{25} dose of drug died and were discarded while those of the other groups survived with different effects on red blood cell AChE activity levels. As shown in **Table 1**, the $LD_{6.25}$ dose likely affected red blood cell AChE activity by not more than 20 %. The residual AChE activity seemed to be at least 80 % of the pre-injection activity level throughout the experiment. In contrast, the $LD_{12.5}$ dose gradually caused accumulating effects and more than 40 % reduction in AChE activity was noted from the 4th week of injection. Therefore, the $LD_{12.5}$ dose was chosen for the study.

Effects of daily injection of dicrotophos at $LD_{12.5}$ dose for 4 weeks

Several effects were observed in the rats injected with a $LD_{12.5}$ (3.75 mg/kg BW) dose of dicrotophos daily. There was fasciculation observed about 20 minutes after the injection which lasted up to 3 hours. The animals were moving around but remained quiet with reduced exploratory behaviors. In addition, increased lacrimation and salivation were also observed. However, they all survived throughout the experiments.

The body weight of the animals in both the saline-injected control group and the dicrotophos-injected group increased, though those of the dicrotophos-injected group did so at a significantly lower rate than the saline-injected group from the 4th day of the injection protocol (**Fig. 2**). There was a lower rate of food intake from the 2nd day for the dicrotophos injection group (**Fig. 3**).

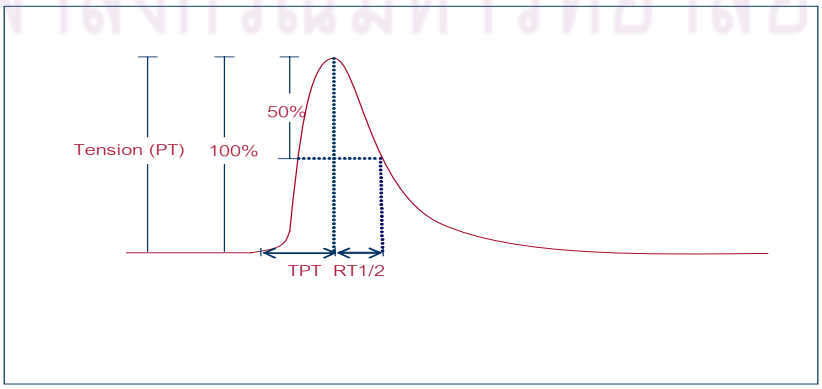

Fig. 1 Myogram of gastrocnemius muscle contraction showing tension or peak twitch tension (PT), time to peak tension (TPT) and one-half relaxation time ($RT_{1/2}$).

Table 1. Effects of daily injection of 2 different doses ($LD_{6.25}$, 1.87 mg/kg BW and $LD_{12.5}$, 3.75 mg/kg BW) of dicrotophos on red blood cell AChE activity (U/L).

Weeks of injection	Red blood cell AChE activity (U/L)	
	$LD_{6.25}$ dose (1.87 mg/kg BW, n=6)	$LD_{12.5}$ dose (3.75 mg/kg BW, n=6)
0	2250.0 ± 113.6 (100.0 %)	2137.0 ± 19.1 (100.0 %)
1	2451.4 ± 235.8 (108.9 %)	2262.0 ± 29.1 (105.7 %)
2	2451.4 ± 525.1 (108.9 %)	2384.0 ± 72.3 (111.5 %)
3	2305.9 ± 293.3 (102.4 %)	1872.0 ± 65.2 (87.6 %)
4	2208.5 ± 182.6 (98.1 %)	1274.0 ± 182.0 (59.6 %)
5	1813.4 ± 63.4 (80.5 %)	1066.0 ± 52.0 (49.9 %)

Values are presented as means ± SE.

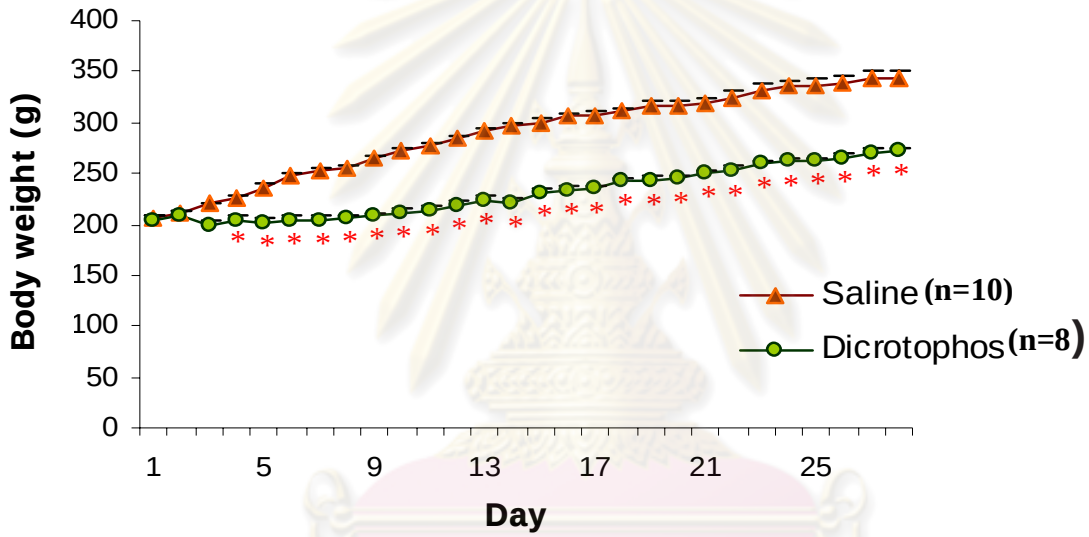

Fig. 2 Effects of multiple doses of dicrotophos on body weight in rats. Values are expressed as means±SE. *significantly different from saline group ($p < 0.05$).

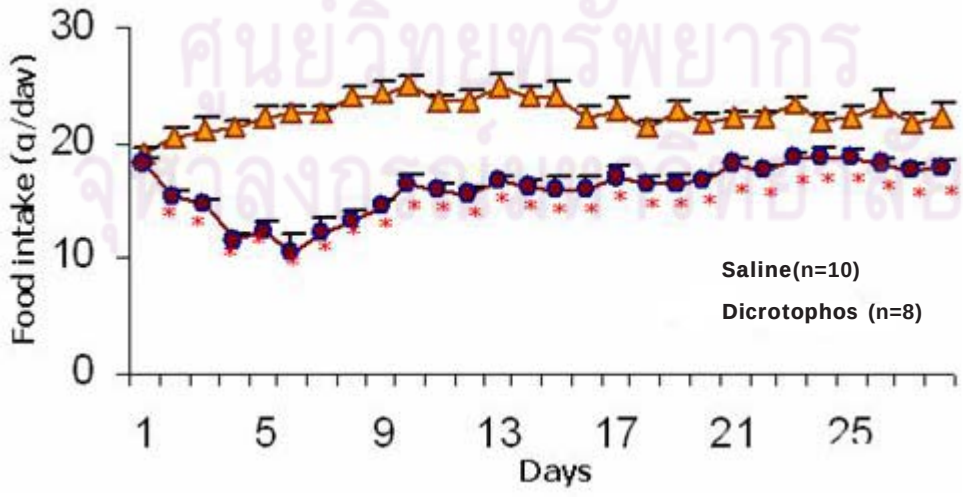

Fig. 3 Effects of multiple doses of dicrotophos on food intake in rats. Values are expressed as means ± SE. *significantly different from saline group ($p<0.05$).

Isometric contractile property

The data from the isometric twitch characteristics (PT, TPT, $RT_{1/2}$) of the gastrocnemius muscle are presented in **Table 2**. Multiple doses of dicrotophos injection caused a significant decrease in PT and TPT but not $RT_{1/2}$, compared to the saline-injected control group.

Acetylcholinesterase activity

The AChE activities in red blood cells and gastrocnemius muscle homogenates after dicrotophos injection are shown. Multiple injections of dicrotophos for 4 weeks significantly decreased AChE activity in both red blood cells and gastrocnemius muscle homogenates compared to the saline control group. The residual AChE activities were decreased to nearly the same levels, about 63-65 % of the saline-injected groups (**Table 3)**.

Discussion

At present, it is established that there are two isoenzymes of cholinesterase in humans. The first of these enzymes is acetylcholinesterase or true cholinesterase found principally in nerve tissue, muscle and red blood cells. This enzyme exhibits specific hydrolytic activity towards the neuro-transmitter acetylcholine [19], but the functions of the enzyme in red blood cells are unknown. The second enzyme, butyrylcholinesterase or BuChE, also known as pseudocholinesterase or plasma cholinesterase, is found principally in plasma and the liver. It has been suggested that this enzyme catalyses hydrolysis in a wide variety of choline and non-choline esters [20], but its exact physiological role is unknown. In the present study, red blood cell AChE activity was used as the index of dicrotophos toxicity, since plasma AChE in rats was reported with inconsistent results [21]. Besides, red blood cell AChE activity was reported to have a slower recovery rate than plasma and brain AChE activity [22]. Therefore, it could be a good index of chronic exposure to any cholinesterase inhibitor after cessation of the exposure.

Dicrotophos is an organophosphate widely used as a pesticide. It inhibits AChE and is therefore acutely toxic due to the increased activation of nicotonic and muscarinic receptors [23]. Delayed toxicity may also occur several days after the acute symptoms which is sometimes called intermediate syndrome [2]. Symptoms may include paralysis of proximal limb muscles, neck flexors and respiratory muscles. Repeated exposure to high organophosphate doses can also lead to long term effects, occasionally affecting behavior as well as mental and visual functions [24]. Based on accumulated data, it is concluded that the mechanism of dicrotophos toxicity in mammals is through inhibition of AChE activity in

Table 2. Effects of daily injection of saline or dicrotophos for 4 weeks on isometric contractile properties of gastrocnemius muscle. PT = peak tension, TPT = time to peak tension, $RT_{1/2}$ = one-half relaxation time.

Variables	Groups of animals	
	Saline-injected (n=10)	Dicrotophos-injected (n=8)
PT (g/g tissue)	62.77 ± 5.80	44.90 ± 3.75**
TPT (ms)	49.33 ± 1.47	44.17 ± 0.83*
$RT_{1/2}$ (ms)	18.00 ± 1.24	20.00 ± 0.89

Values are presented as means ± SE; *significantly different from saline-injected group ($p < 0.05$); **significantly different from saline-injected group ($p<0.01$).

Table 3. Effects of daily injection of saline or dicrotophos for 4 weeks on AChE activity in red blood cell and gastrocnemius muscle homogenates.

Groups	AChE activity in red blood cell (U/L)	AChE activity in muscle homogenate (U/L/g tissue)
Saline-injected (n=10)	2514.4 ± 104.1 (100.0 %)	637.1 ± 41.5 (100.0 %)
Dicrotophos-injected (n=8)	1637.4 ± 91.0 ** (65.1%)	405.8 ± 26.9 ** (63.6 %)

Values are expressed as means±SE; **significantly different from saline-injected group ($p<0.01$).

nerve tissue. Therefore, the inhibition of AChE in the brain is identified as the most sensitive index of the toxic effect of dicrotophos in animals. However, in human studies, since brain AChE levels cannot be examined, red blood cell AChE levels are used instead to assess the health risk of the exposure in humans [25].

In humans, signs and symptoms of organophosphate exposure result from the inhibition of the AChE enzyme in both the central and peripheral nervous systems [26]. Typical cholinergic symptoms include flaccid skeletal muscle of paralysis, total inhibition of plasma and red blood cell AChE activity [6]. In animals, brain AChE may be inhibited, even though neuropathological examination may not show any abnormalities [27, 28].

A long term toxicity test in animals showed that high-dose exposure to dicrotophos affected mean body weight and food intake. Those effects occurred in both male and female animals. However, by gross examination, no changes in absolute and relative organ weights were found. Increased white blood cell count was noted in addition to other cholinergic signs [28]. In the present study, body weight gain and food intake in rats exposed to multiple injection of dicrotophos were significantly retarded compared to the corresponding saline-injected animals. Both were affected at or during nearly the same period of dicrotophos injection. These results agree with those obtained from both short term and long term toxicity tests in animals. In rats, most of the studies showed that dicrotophos exposure caused decreases in body weight gain and food intake in both sexes. The animals showed tremors and decreases in red blood cell AChE, plasma AChE and brain AChE, though there were no dose-related changes in internal organ weights [27-29]. However, there are few studies with no effect on body weight from dicrotophos drug treatment. Notably those studies with no changes used dermal exposure [21].

The present study showed that daily injections of dicrotophos for 4 weeks caused a marked and significant decrease of AChE activity in RBC. The same level of AChE decrease was observed from AChE activity in gastrocnemius muscle homogenates from rats treated with dicrotophos. The results indicate that dicrotophos might cause motor end plate damage and thus loss of AChE activity, which has apparently never been reported before. This finding agrees with a study in which another OP, metylparathion, was injected into rats for 4 weeks. It was observed that the drug decreased the red blood cell level of AChE activity, and the levels correlated with motor end plate sustained AChE activity [14].

The characteristics of skeletal muscle activity depend on neurotransmitter ACh release and muscle electrochemical activity [30]. The changes in neuromuscular activity may lead to alteration in muscle metabolism, contractile properties and neuromuscular transmitters. The results from the present study demonstrated that after multiple injection of dicrotophos for 4 weeks, there were significant decreases in both PT and TPT but not in $RT_{1/2}$ which was likely increased though not statistically significant. The results indicate that the 4-week prolonged dicrotophos exposure might cause a decrease of Ca^{2+} release from SR and hence the muscle twitch tension. Generally, the twitch characteristics of PT, TPT and $RT_{1/2}$ are indicative of Ca^{2+} handling by the muscle. TPT is the amount of time it takes the muscle to develop PT and is generally indicative of Ca^{2+} release by the sarcoplasmic reticulum. The $RT_{1/2}$ is the time it takes for PT to return to half of the force generated during the peak isometric twitch after removal of the stimulus. This $RT_{1/2}$ value is generally thought to be indicative of Ca^{2+} uptake by the sarcoplasmic reticulum [31]. The reduced twitch characteristics could result from deleterious effects producing a reduced Ca^{2+} release from the sarcoplasmic reticulum, changes in the regulatory proteins, and/or direct effects acting at the cross bridge [32]. As previously shown, the decrease of myoplasmic Ca^{2+} level due to an impairment of Ca^{2+} release was considered as one of the major causes of the decline in force [33]. Therefore, the decreases in PT after multiple dicrotophos exposure in the present study might be due to a decrease in Ca^{2+} release from the sarcoplasmic reticulum. Supporting this assumption are the findings that a progressive reduction in myoplasmic Ca^{2+} concentration accompanied the loss of muscle tension and the twitch duration is dependent on the time course of the increase in intracellular Ca^{2+} [34]. The previous study of Panenic et al [35] in which fatigability of muscle after AChE inhibition in rats was reported, and the findings that nerve agents caused muscle weakness [36] also support the assumption.

In many cases of neuropathic organophosphate poisonings, the residual effects on hand strength were found with muscle weakness which may last for several months or years [37-39]. The muscle

weakness cannot be explained on the basis of nervous tissue AChE inhibition alone and various possible causes have been proposed. These include necrotizing myopathy and neuromuscular block [2, 3, 12, 40, 41], damage to the peripheral nerves [42], and desensitization and down regulation of ACh receptors [13, 43, 44]. Excessive calcium in nerve endings leading to localized muscle injury is another possible contributor to muscle weakness caused by action of the organophosphate [45]. In the absence of further study to elucidate the exact mechanisms, the decrease in peak tension observed in the present study after prolonged dicrotophos exposure might be due to any of the aforementioned causes, each having the same degree of possibility.

In conclusion, prolonged exposure to dicrotophos affected skeletal muscle contractile characteristics and a decrease in peak tension was observed. The results confirmed the muscle weakness in humans resulting from organophosphate exposure.

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References

1. Lotti M. Treatment of acute organophosphate poisoning. Med J Aust. 1991;154:51-5.
2. Senanayake N, Karalliedde L. Neurotoxic effects of organophosphorus insecticides. An intermediate syndrome. N Engl J Med. 1987;316:761-3.
3. De Bleecker J, Van den NK, Colardyn F. Intermediate syndrome in organophosphorus poisoning: a prospective study. Crit Care Med. 1993;21:1706-11.
4. Moretto A. Organophosphorus insecticides: toxicological issues [online]. 2005 [cited 2005 Jan 17]. Available from: http://www.icps.it/english/bollettino.psn98/980201.html
5. Lotti M. The pathogenesis of organophosphate polyneuropathy. Crit Rev Toxicol. 1991;21:465-87.
6. Health Council of the Netherlands: Committee on Updating of Occupational Exposure Limits. Dicrotophos. Health-based Reassessment of Administrative Occupational Exposure Limits. 2000/15OSH/069 [online]. The Hague: Health Council of the Netherlands; 2003 [cited 2005 May 17]. Available from: http://www.gr.nl/pdf.php?ID=752&p=1.html
7. Costa LG. Current issues in organophosphate toxicology. Clin Chim Acta. 2006;366:1-13.
8. Ray D. Organophosphorus esters: an evaluation of chronic neurotoxic effects. Institute for environment and health [online]. 1998 [cited 2005 May 17]. Available from:http://www.silsoe.cranfield.ac.uk/ieh/pdf/sr5.pdf.html
9. Kelly SS, Mutch E, Williams FM, Blain PG. Electrophysiological and biochemical effects following single doses of organophosphates in the mouse. Arch Toxicol. 1994;68:459-66.
10. Kelly SS, de Blaquiere GE, Williams FM, Blain PG. Effects of multiple doses of organophosphates on evoked potentials in mouse diaphragm. Hum Exp Toxicol. 1997;16:72-8.
11. de Blaquiere GE, Williams FM, Blain PG, Kelly SS. A comparison of the electrophysiological effects of two organophosphates, mipafox and ecothiopate, on mouse limb muscles. Toxicol Appl Pharmacol. 1998;150:350-60.
12. Mense S, Simons DG, Hoheisel U, Quenzer B. Lesions of rat skeletal muscle after local block of acetylcholinesterase and neuromuscular stimulation. J Appl Physiol. 2003;94:2494-501.
13. Katz EJ, Cortes VI, Eldefrawi ME, Eldefrawi AT. Chlorpyrifos, parathion, and their oxons bind to and desensitize a nicotinic acetylcholine receptor: relevance to their toxicities. Toxicol Appl Pharmacol. 1997;146:227-36.
14. Thongchuai B. Effects of Zingiber officinale Roscoe on methyl parathion intoxication in rats [MS Thesis]. Chiang Mai: Chiang Mai University; 2004.
15. Kessomboon P. Sickness of Thai people due to pesticides. In: Proceedings for the National Health Council Meeting. 2003. p. 13-9.
16. Banned pesticides [online]. 2003 [cited 2005 Mar 2]. Available from: http://www.ipmthailand.org/th/pesticide/pesticides_banned. html
17. True BL, Dreisbach RH. Cholinesterase inhibitor pesticides. In: True BL, Dreisbach RH, editors. Dreisbach's handbook of poisoning. 13th ed. London: Parthenon Publishing; 2002. p. 123-31.
18. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.
19. Alles GA, Hawes RC. Cholinesterase in the blood of man. J Biol Chem. 1940;133:275-390.
20. Mendel B, Rudney H. On the type of cholinesterase present in the brain tissue. Science. 1943;98:201-2.

21. Hageman. 28 day repeated dose dermal toxicity study in the rat. Test No. 911267 C1414 tech. Final Report. Ciba Geigy [online]. 1992 [cited 2005 May 17]. Available from:http://www.apvma.gov.au/chemrev/monotoxx2.pdf.html
22. Hend RW, Brown VKH. A reversibility study on cholinesterase activity in rats fed AZODRIN for 8 weeks. Shell Research Ltd, Sittingbourne. TLGR. 79.154. In: National Registration Authority for Agricultural and Veterinary Chemicals, Australia. Section 4 [online]. 1981 [cited 2005 May 17]. Available from: http://permits.nra.gov.au/chemrev/monotox.pdf.html
23. Koelle GB. Pharmacology of organophosphates. J Appl Toxicol. 1994;14:105-9.
24. Eyer P. Neuropsychopathological changes by organophosphorus compounds-a review. Hum Exp Toxicol. 1995;14:857-64.
25. Jeyaratnam J, Maroni M. Organophosphorous compounds. Toxicology. 1994;91:15-27.
26. Keifer MC, Mahurin RK. Chronic neurologic effects of pesticide overexposure. Occup Med. 1997;12: 291-304.
27. Hazard Identification Assessment Review Committee. Hazard assessment of the organophosphates. Washington DC, USA: US Environmental Protection Agency, Office of Pesticide Programs, Health Effect Division [online]. 1998 [cited 2005 May 17]. Available from: http://www.epa.gov/pesticides/op/dicrotophos.html
28. Hrdy DE. Dicrotophos (chemical NO: 035201; List A, Reregistration case NO. 0145). HED revision to risk assessment for reregistration eligibility document (RED). Washington DC, USA: US Environmental Protection Agency, Health Effects Division [online]. 1999 [cited 2004 Apr 26] Available form: http://www.epa.gov/pesticides/op/dicrotophos.html
29. Howard DJ, Donoso J, Johnston CD. Bidrin. Safety evaluation by a chronic feeding study in the rat for two years. Final report. Herndon VA: Woodard Research Corporation, 1967.
30. Fernandez HL, Donoso JA. Exercise selectively increases G4 AChe activity in fast-twitch muscle. J Appl Physiol. 1988;65:2245-52.
31. Eason JM, Dodd SL, Powers SK, Martin AD. Detrimental effects of short-term glucocorticoid use on the rat diaphragm. Phys Ther. 2000;80:160-7.
32. Fitts RH. Cellular mechanisms of muscle fatigue. Physiol Rev. 1994;74:49-94.
33. Germinario E, Esposito A, Megighian A, Midrio M, Betto R, Danieli-Betto D. Effects of modulators of sarcoplasmic Ca2+ release on the development of skeletal muscle fatigue. J Appl Physiol. 2004;96: 645-9.
34. Blinks JR, Rudel R, Taylor SR. Calcium transients in isolated amphibian skeletal muscle fibres: detection with aequorin. J Physiol. 1978;277:291-323.
35. Panenic R, Gisiger V, Gardiner PF. Fatigability of rat hindlimb muscles after acute irreversible acetylcholinesterase inhibition. J Appl Physiol. 1999; 87:1455-62.
36. Hulet SW, McDonough JH, Shih TM. The dose-response effects of repeated subacute sarin exposure on guinea pigs. Pharmacol Biochem Behav. 2002;72: 835-45.
37. Karalliedde L, Senanayake N. Organophosphorus insecticide poisoning. Br J Anaesth. 1989;63:736-50.
38. Karalliedde L, Henry JA. Effects of organophosphates on skeletal muscle. Hum Exp Toxicol. 1993;12:289-96.
39. Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. Chronic neurological sequelae to organophosphate pesticide poisoning. Am J Public Health. 1994;84:731-6.
40. He FS. Acute organophosphate poisoning induced intermediate myasthenia syndrome. Chin J Ind Hyg Occup Dis. 1996;14:257-8.
41. He F, Xu H, Qin F, Xu L, Huang J, He X. Intermediate myasthenia syndrome following acute organophosphates poisoning-an analysis of 21 cases. Hum Exp Toxicol. 1998;17:40-5.
42. Miranda J, McConnell R, Wesseling C, Cuadra R, Delgado E, Torres E, et al. Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticides. Occup Environ Med. 2004;61:e4.
43. Xu H, He X, Xie Z, He F. Blocking effects of dimethoate on acetylcholine receptor channels. Wei Sheng Yan Jiu. 1997;26:154-8.
44. Yang D, Niu Y, He F. Functional changes of nicotinic acetylcholine receptor in muscle and lymphocyte of myasthenic rats following acute dimethoate poisoning. Toxicology. 2005;211:149-55.
45. Baker DJ, Sedgwick EM. Single fibre electromyographic changes in man after organophosphate exposure. Hum Exp Toxicol. 1996;15:369-75.