PESTICIDE EXPOSURE IN TREE PLANTERS

by

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ABSTRACT

Tree planters in British Columbia are exposed to a low level of pesticide residue on the seedlings that they plant. Previously reported symptoms and anecdotal evidence indicated the possibility of a significant effect from absorption of organophosphate and/or carbamate pesticide on some tree planters. The intent of this study was to use physiological and biochemical indices of a pesticide-related toxicological effect to establish or refute the existence of a toxicological hazard to tree planters from their chronic exposure to a low level of pesticide. An attempt was made to correlate any observed effect of pesticide absorption on these indices with individual parameters of pesticide exposure and with the number of health complaints reported by a tree planter. Prework and postwork erythrocyte acetylcholinesterase (AChE), serum pseudocholinesterase (PChE), and total whole blood cholinesterase (WChE) activity were serially determined in tree planters throughout a tree planting season to identify any acute or developing pesticide-related effect. An association was found between inhibition of the above cholinesterases and several exposure parameters, which included: the interval between the time of application of a carbamate pesticide to a seedling and the time of planting (CBRE), the interval between the time of application of an organophosphate pesticide and cold storage of a seedling (OPCLD), and the productivity of an individual worker. It was determined that the degree of acute PChE and WChE inhibition could be related to the number of general health complaints (general HSUM), which included coughing, nose and eye irritation, skin rash, headache, and sore throat. A significant inhibition of PChE developed throughout a tree planting season was also identified. A tree planter's ulnar and median motor nerve conduction velocity and latency was found to be within the normal range at the end of a season of tree planting, and no significant change in these measures was found upon retesting after several months of recovery. Several suggestions were presented to minimize both a tree planter's daily absorption of pesticide and the chronic effect of low-level pesticide absorption.

DEDICATION

This thesis is dedicated to the many tree planters who believed in what we set out to accomplish and who repeatedly volunteered their blood, sweat, and time, despite the extreme conditions of temperature, rain, snow, and black flies.

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INTRODUCTION

Exposure to pesticides is a hazard of significant concern to the health and well-being of people whose occupation involves the use or handling of these chemicals. It has been estimated that as many as one million poisonings and 80,000 fatalities occur worldwide each year as a result of pesticide exposure (Goulding, 1988; Bull, 1982). A disproportionate number of such poisonings occur in the third world or developing countries where both agricultural regulations and worker awareness are lacking (Xue, 1987). Farmer education and regulation of pesticide use was shown to reduce the incidence of pesticide poisoning from 4.5% to 0.1% during a two year period, in spite of a 13 fold increase in pesticide use (Shih *et. al.*, 1985). This case serves to demonstrate the utility of research focussed on recognizing and characterizing an exposure-related problem in order to minimize the health risk resulting from ignorant or negligent use of a hazardous substance.

It has been established that certain biochemical and physiological effects of specific classes of pesticide may be used as markers for significant pesticide exposure and absorption in humans. In particular, depression of erythrocyte acetylcholinesterase and serum cholinesterase activity have both been used as a sensitive indicator of exposure to organophosphate and carbamate pesticide once a baseline activity level has been determined for a worker (Trundle and Marcial, 1988; Magnotti *et. al.*, 1988; Vandekar, 1980; Derache, 1977; Callaway *et. al.*, 1951). Even in the absence of a baseline value, the observation of trends in serially determined cholinesterase activity has proven effective in confirming organophosphate poisoning clinically (Coye *et. al.*, 1987; Midtling *et. al.*, 1985). California law requires cholinesterase monitoring for workers involved in the formulation, mixing, loading or application of organophosphate pesticides. Detection of a 30% depression of serum cholinesterase activity warrants an investigation of working conditions

and detection of 50% depression of serum cholinesterase warrants removal of an individual from a contaminated work site (Magnotti *et. al.*, 1988; Popendorf and Leffingwell, 1982).

Erythrocyte acetylcholinesterase and serum cholinesterase are isozymes of neuronal acetylcholinesterase which catalyzes the hydrolysis of acetylcholine released into the synapse of nicotinic or muscarinic neural pathways. When acetylcholinesterase is inhibited, as in organophosphate poisoning, acetylcholine accumulates in the synapse, altering neural transmission and control of the innervated organ (Derache, 1977). Physical manifestation of this altered neural control accounts for much of the acute symptomology associated with pesticide intoxication, namely: sweating, miosis, salivation, vomiting, abdominal pain, diarrhea, altered heart rhythm, muscle twitching and weakness, headache, dizziness, nausea, and in the extreme case loss of consciousness (Cessna, 1988; Costa, 1988; Peiris *et. al.*, 1988; Morgan, 1980). Low level chronic exposure to an anticholinesterase agent results in the development of tolerance to the symptoms listed above, possibly due to a decrease in the density of both nicotinic and muscarinic receptors in the peripheral and/or central nervous system. In this case, acetylcholinesterase activity may be depressed to a very low level without ensuing peripheral symptomology (Costa, 1988; Aas *et. al.*, 1987; Dille and Smith, 1964). It has been postulated that the severity of peripheral symptomology is related more to the rate of cholinesterase depression than to the absolute level of activity (Trundle and Marcial, 1988; Aas *et. al.*, 1987; Coye *et. al.*, 1987; Sumerford *et. al.*, 1953). Lack of symptomology in an individual should not, however, be taken to indicate that the individual is immune to an anticholinesterase effect, since it has also been noted that a cholinesterase-inhibited individual is more susceptible to a subsequent acute exposure to an anticholinesterase agent than a healthy individual (Coye *et. al.*, 1987).

It has been demonstrated that prolonged exposure to an anticholinesterase chemical can produce peripheral myopathy (Fenichel *et. al.*, 1972; Wecker *et. al.*, 1978), neuropathy and disruption of the neuromuscular junction (Senanayake and Karalliedde, 1987; Engel *et.*

al., 1973). Histological analysis of the neuromuscular junction has indicated destruction of some postsynaptic folds, resulting in an increased synaptic width (Engel *et. al.*, 1973). Several authors have reported a significant negative change in electrophysiological activity in workers exposed to an anticholinesterase chemical compared with a control group, suggesting that EMG measurement may be useful in monitoring pesticide exposure (Misra *et al.*, 1988; Wadia *et. al.*, 1987; Ring *et. al.*, 1985; Hussain *et al.*, 1981; Drenth *et. al.*, 1972; Jager *et al.*, 1970). Jager *et al.* (1970) found EMG analysis to be more sensitive to anticholinesterase exposure than was cholinesterase determination. The fact that these non-invasive techniques may be indicative of pesticide-induced damage warrants further investigation into their potential for monitoring pesticide exposure.

Many of the peripheral and central symptoms associated with organophosphate and carbamate poisoning are commonly reported health complaints of tree planters in British Columbia (Banister *et. al.*, 1990; Smith, 1987). While it is difficult to establish a complete etiology of such symptoms, pesticide exposure may not be ruled out. Symptoms of low level pesticide intoxication may resemble influenza or mild heat exhaustion (Spear *et. al*, 1975). Giardiasis ("beaver fever"), salmonella, and campylobacter are other possible causes of intestinal symptoms given the existing relatively primitive camp conditions of tree planters (Smith, 1987; Vancouver Sun, 1986; Dar Lin, 1985).

The use of pesticides in silviculture is an obvious possible toxicological hazard to a tree planter which may be related to some of the observed health complaints in the industry. Worker concern and the anecdotal association of health complaints with possible pesticide exposure in tree planting work is evident in the popular press (Vancouver Sun, 1981; Vancouver Sun, 1986) and trade magazines (Screef, 1989); however, there is very little documented scientific literature specifically addressing the issue. Apol and Thoburn (1976 a-f) reported an association between systemic symptoms and tree planter exposure to Thiram, but the association could only be drawn from a general medical history.

Attribution of symptoms to Thiram exposure was based on a high pesticide application rate to the seedlings planted and long work weeks in the field.

Tree seedlings planted in British Columbia are typically treated with both insecticides and fungicides during nursery growth (Nursery, 1987). It is generally assumed that the amount of pesticide residue remaining on a seedling at the time of planting has diminished to an insignificant level, and that a tree planter is exposed only to an acceptable residual level of pesticide during his/her handling and planting of seedlings. This is a basic premise of the so-called reentry principle used to estimate the time (reentry interval) between pesticide application and safe reentry of a field worker into a treated area. A similar principle can be applied to estimate a time interval between the time of application of a pesticide to tree seedlings and the time of planting those seedlings. The longer the reentry interval, the lower the concentration of pesticide residue on the seedling. A reentry model can be developed to estimate an appropriate reentry interval, after which the pesticide residue on a seedling would be considered safe for handling.

A reentry model is based on estimated pesticide residue decay rate and an acceptable exposure level or physiological response of the worker to a known pesticide dose. Practical use of such a model is intended to protect a field worker or harvester from entering a field too soon after crop treatment, thus exposing themselves to a significant pesticide hazard (Popendorf and Leffingwell, 1982; Popendorf, 1985). In the past, field workers and crop harvesters have been subject to significant pesticide exposure from pesticide residue on foliage and soil (Iwata *et. al.*, 1977; Spencer *et. al.*, 1977; Zweig *et. al.*, 1985).

Industrial hygiene studies of the working conditions of tree seedling nursery employees and treeplanters exposed to particular fungicides have found an acceptable level of residue on seedlings, and acceptable dermal and inhaled exposure dose (B.C. Research, 1988; 1986). It has been noted, however, that a "pharmacologically significant absorption may

result from seemingly trivial exposure" (Morgan, 1980, p.98) and that the exposure level varies greatly among treeplanters (B.C. Research, 1988).

During pilot studies for this thesis, serum cholinesterase activity was measured before and after daily tree planting in a selected group of workers. The tests showed that several treeplanters had depressed serum cholinesterase activity after one day's work, while others, similarly exposed, were apparently unaffected (Banister *et. al*, 1990). The factors contributing to depressed cholinesterase in affected treeplanters are still unknown and warrant further analysis.

Several aspects of hygienic practice, in theory, could account for the variability in pesticide exposure and absorption between individuals, and therefore account for the observed interindividual variability in cholinesterase depression. It has been demonstrated by several authors that conscientious hygienic practice, work habits, and the use of protective clothing significantly decrease the dermal absorption and inhalation of pesticide by an individual (Mcdonald *et. al*, 1988; Cessna, 1988; B.C. Research, 1988; Raheel, 1987).

As noted earlier, significant absorption may occur from a seemingly trivial exposure (Morgan, 1980). In the case of tree planters, where adequate hygiene is impractical under prevailing field conditions, this fact is very relevant. The intention of this investigation, therefore, is to use inhibition of serum cholinesterase activity in a group of tree planters as a marker of daily pesticide absorption, and erythrocyte cholinesterase inhibition as a marker of the chronic low level of pesticide absorption which results in a significant cumulative erythrocyte cholinesterase inhibition. Further, it is intended to correlate these measures with various field measures of possible pesticide exposure, including: health complaints, pesticide applied to the seedlings, time between pesticide application and planting, and productivity of the individual planter. Inhibition of motor nerve conduction velocity after prolonged tree planting activity will be examined as a possible physiological marker of pesticide absorption.

I. TOXICOLOGY OF PESTICIDES

ORGANOPHOSPHATES

The organophosphate insecticides comprise alkyl phosphates and aryl phosphates, including a range of chemical modifications produced by amination and thiol sulphonation of the respective basic molecules. For a review of specific structures see Derache (1977). Most organophosphates are readily absorbed through skin and mucous membranes.

Organophosphate pesticides exert their effect in both insects and mammals primarily through influence on cholinergic neurotransmission. In humans, the effects are fairly wide spread since the cholinergic synapse is involved in neuromuscular transmission, sympathetic and parasympathetic autonomic function, as well as central functioning in many brain regions (Trundle and Marcial, 1988; Ganong, 1983). Organophosphates inhibit synaptic and neuromuscular acetylcholinesterase activity, resulting in an accumulation of acetylcholine within the synapse. Elevated synaptic acetylcholine produces a more intense and prolonged postsynaptic response. It is believed that the majority of acute and chronic symptoms are due to this latter occurrence (Morgan, 1980). In acute poisoning, symptomatic expression of altered neural control is vagomimetic, including: nausea, vomiting, abdominal pain, rhinorrhea, excessive salivation, bronchospasm, increased bronchial secretion, hypotension with bradycardia, and miosis (Milby, 1971). The neuromuscular effects result in muscular weakness, twitching, cramping, and in the severest case, paralysis. Centrally mediated effects involve fatigue, malais, headaches, anorexia, and coma (Coye *et. al.*, 1987; Derache, 1977). Katz and Marquis (1989) suggest that basing organophosphate toxicity solely on AChE effects is erroneous, since both a direct interaction with muscarinic receptors and an interplay with other neurotransmitter systems have been demonstrated in low level chronic exposure of rats to organophosphate. These actions would influence both central and peripheral symptomology. Das Gupta *et. al.* (1989) found that organophosphate depression of monosynaptic reflex was independent

of cholinesterase inhibition, and was probably due to a direct interaction with muscarinic receptors.

Acute organophosphate poisoning is usually from suicide attempts or inadvertent over-exposure, although several cases resulting from occupational exposure have been documented (e.g. Brown *et. al.*, 1989; Karalliedde and Senanayake, 1988; Coye *et. al.*, 1987; Xue, 1987). Reports of organophosphate poisoning from chronic exposure are less evident in the literature; however, chronic effects have been studied in myasthenia gravis and glaucoma patients who are treated with anticholinesterase drugs (Engel *et. al.*, 1973; Fenichel *et. al.*, 1972) as well as in crop dusters who apply organophosphate pesticide to fields (Dille and Smith, 1964). Chronic effects of organophosphate poisoning include memory impairment, myopathies and neuropathies, as well as behavioural dysfunction.

In addition to their cholinergic effect, hematological studies have reported a variety of blood dyscrasias due to organophosphate or carbamate exposure in humans. Agranulocytosis, aplastic anemia, and hypoplastic anemia have been reported due to both organophosphate and carbamate exposure (David and Fairchild, 1980). Chronic exposure of an individual in an occupational setting and of larger populations exposed to an extensive spraying program have demonstrated leukopenia and diminished hemoglobin compared with control populations (Davignon *et. al.*, 1965; Jegier, 1964). The exposure dose in all these studies has been estimated at one percent of a no effect level (David and Fairchild, 1980). This supports Morgan's (1980) observation that a seemingly trivial exposure may be pharmacologically significant. It has been postulated by David and Fairchild (1980) that the change observed in hematological parameters after organophosphate exposure in humans is due to altered hepatic function, and that certain individuals show a hypersensitivity to organophosphate pesticide exposure.

Organophosphates have been shown to alkylate a variety of biological compounds quite readily, including amino acids, nucleic acids, amines and hydroxyls (Derache, 1977). Although no mutagenic or teratogenic effects have been demonstrated from

organophosphate ingestion in animals except after an exceedingly large dose, this alkylating ability may be one method by which organophosphates could exert a mutagenic or teratogenic effect.

Due to their relatively rapid rate of catabolism, most organophosphates do not accumulate significantly in human tissue; however, some arylphosphates such as diazinon have been observed to accumulate in adipose tissue (Derache, 1977). In other tissue, organophosphates are readily metabolized by several esterases, phosphatases, microsomal oxidases or amidases. Species differences exist in the ability to metabolize certain organophosphates, therefore, metabolic products and their specific effects differ from species to species.

Some metabolic products are more toxic than the organophosphate insecticide itself. Classical examples of this are parathion and malathion which are oxidized by liver microsomal enzymes to paraoxon and malaoxon, respectively. Milby *et. al* (1964) reported an outbreak of clinical illness due to parathion residue among orchard workers, even though the parathion exposure level appeared to be within acceptable standards. Illness was attributed to the breakdown product paraoxon. Paraoxon and malaoxon are much stronger cholinesterase inhibitors than the parent compounds.

CARBAMATES

N-methylcarbamate pesticides are generally used as fungicides. They are readily absorbed through the skin and mucous membranes. N-methylcarbamate is a reversible inhibitor of cholinesterase activity, exerting its effect on humans through a mechanism identical to that of an organophosphate. This mechanism involves an accumulation of acetylcholine in the synapse due to AChE inhibition, resulting in altered neuro-neuronal and neuromuscular communication. In addition, Das Gupta *et. al.* (1989) studied a concentration-dependent depression of the monosynaptic reflex in neonatal rats, which was found to be independent of AChE inhibition. These authors also reported a direct effect of

the carbamates pyridostigmine and physostygmine on the neuromuscular junction which was not cholinesterase related. Pyridostigmine was found to be a weak agonist of ion channels in nicotinic reception, while physostigmine was found to block ion channels in the open conformation. These properties have been applied to the treatment of myasthenia gravis (Engel *et. al.*, 1973; Fenichel *et. al.*, 1972; Roberts and Wilson, 1969). Carbamates have also been implicated in the production of blood dyscrasias, as discussed above (David and Fairchild, 1980).

Carbamates are detoxified in human tissue by several enzyme systems including esterases and mixed function oxidases (Takahashi *et. al.*, 1987). The reversible nature of carbamate-induced AChE inhibition has been used as a protective mechanism against the more severe irreversible inhibition resulting from organophosphate absorption (Das Gupta *et. al.*, 1989).

NEUROPATHOLOGICAL EFFECTS OF ORGANOPHOSPHATE AND CARBAMATE CHEMICALS

Central Effects

Central neurotoxic effects have been described in the literature, including effects resulting from chronic low-level exposure to an organophosphate chemical. These effects may be related to some of the behavioral and psychological symptoms that are generally associated with exposure to anticholinesterase chemicals.

It was observed by Aas *et. al.* (1987) that acute exposure to soman resulted in 90% inhibition of AChE in rat brain hippocampus, however no inhibition was found elsewhere in rat brain. Veronesi *et. al.* (1990) observed histopathological changes in rat hippocampus after long-term low-level exposure to the organophosphate Fenthion. Histopathology consisted of neural necrosis, swelling, gliosis, and cell dropout in several regions of hippocampus after as little as two months exposure. Acetylcholinesterase activity in the hippocampus was depressed 65% at this time and 85% after ten months exposure. Hippocampal muscarinic receptor binding was also reduced. Aged rats showed more

severe hippocampal damage than young adult (two month old) rats, however both groups of animals demonstrated serious neurotoxic effects on the hippocampus after low-level anticholinesterase exposure. The hippocampus is directly associated with learning and memory, and plays a role in limbic functions such as motivation and control of hypothalamic regulation of autonomic nervous and hormonal activity. This demonstrates the potential for serious neurological consequences after low level organophosphate exposure during a period of time that is similar to the duration of a tree planting season.

Peripheral Axonal Effects

Peripheral axonal effects due to anticholinesterase pesticide absorption may be grossly divided into two categories: organophosphorus-induced delayed polyneuropathy (OPIDP), and less severe neurotoxic effects with no delay in onset. Several investigators have reported reversible peripheral axonal effects such as slowing of conduction velocity and longer distal latency in workers exposed to pesticide (Prinsen and Van Sittert, 1980; Ring *et. al.*, 1985; Misra *et. al.*, 1988) and in patients who have attempted suicide through ingestion of pesticide (Wadia *et. al.*, 1987). Although longer distal latency may be due to a neuromuscular junction dysfunction, latency research has been included in this section of the literature review because of its direct relationship to conduction velocity. Delayed neurotoxic effects will be briefly introduced to illustrate the magnitude of toxicity that is sometimes related to organophosphate exposure.

Some organophosphates exhibit a neurotoxic effect which does not appear for one to three weeks after exposure, by which time the cholinesterase concentration may have returned to normal. This effect has been called delayed neurotoxicity or "organophosphorus-induced delayed polyneuropathy" (OPIDP) and may result in permanent neurological damage with degeneration of distal motor axons and the anterior columns of the thoracic and lumbar spinal cord (Wadia *et. al.*, 1987; Lotti *et. al.*, 1984).

Senanayake and Johnson (1982) have presented several case-studies of individuals showing delayed neuropathy after methamidophos (Tamaron) ingestion or inhalation. They reported severe weakness of the distal musculature developing two to four weeks after contact with the pesticide, as well as an increased tone of proximal muscle and an exaggerated tendon reflex response. These authors reported that electromyography was indicative of distal muscle denervation .

Pesticide-induced OPIDP generally follows acute symptoms of cholinesterase inhibition, however, the polyneuropathy is not related to the anticholinesterase effect. Phosphorylation of a neurotoxic esterase and "aging" of the phosphoryl-enzyme complex are required to produce the delayed neurotoxic effects (Lotti *et. al.*, 1984). An *in vitro* test has been developed using inhibition of neurotoxic esterase activity to distinguish organophosphates that may cause OPIDP from those that do not (Lotti *et. al.*, 1984). Pesticides typically used by B.C. Nurseries are not associated with OPIDP.

An altered interaction between Schwann cells and motor neurons has been observed in rats following chronic low-level administration of prostigmine, an anticholinesterase carbamate drug used in the treatment of myasthenia gravis (Engel *et. al.*, 1973). This indicates the possibility of a decreased motor nerve conduction velocity due to a change in the myelination of a peripheral motor axon.

Prinsen and Van Sittert (1980) found a significant decrease, although still within the normal range, for the mean in a group of spraymen (n=7) of ulnar motor nerve conduction velocity, median sensory nerve conduction velocity, ulnar slow fiber conduction velocity, and corneal reflex after an eight month season of spraying a synthetic pyrethroid. No significant group effect was demonstrated after a single day of spraying, however, some individuals did show a significant depression within the normal range. No recovery data were published.

A correlation between certain EMG parameters and the presence of mild clinical symptoms of organophosphate intoxication was found by Ring *et. al.* (1985) in a group of

30 crop spray workers. These authors measured 16 EMG parameters, of which 5 correlated with symptom occurrence. Conduction velocity of the lateral popliteal motor nerve and the ulnar motor nerve above the elbow were found to be significantly lower in individuals exibiting clinical symptoms of exposure. Motor distal latency of the median and ulnar nerves, and sensory distal latency of the ulnar nerve were found to be significantly longer in affected individuals. All EMG measures were, however, within the normal range. Further, it was noted that there was a correlation between the status of the above EMG measures at the beginning of a spraying season and the development of symptoms both during the current and the following spraying season. Thus it was possible to identify certain individuals within a work force who show increased sensitivity to organophosphate pesticide exposure and who are most likely to become intoxicated, even from low level exposure. One in eight of the 30 spraymen studied showed a predisposition to symptom development from exposure to low level concentration of organophosphate pesticide, based on an EMG measurement at the start of the spraying season.

Wadia *et. al.* (1987) studied 2000 patients suffering acute high level organophosphate intoxication after attempting suicide. These individuals showed progressively slower ulnar motor nerve conduction velocity as the severity of poisoning increased, although nearly all conduction velocities measured were within the normal range and the slowest velocity was 47 m/sec.

Twenty-nine percent of workers exposed to a repeated low dose of fenthion showed a significant reversible effect on peroneal motor nerve conduction velocity and distal latency, median nerve distal latency, F-minimal latency, and H-reflex latency (Misra *et. al.*, 1988). These subjects also showed significant depression of serum PChE activity which then rose 23% during a three week recovery period.

The above finding that a significant proportion of individuals who have been exposed to an organophosphate or carbamate chemical exibit decreased conduction velocity across an axonal segment suggests either a change in the axonal membrane properties or a change

in the status of myelination as a result of chemical exposure. Serial electrophysiological monitoring of workers who are exposed to these chemicals, therefore warrants further exploration as an early indicator of pesticide-induced pathology. The increased distal motor nerve latency also observed in organophosphate or carbamate exposed individuals may reflect either a peripheral axonal deficiency or a disruption of the neuromuscular junction.

Neuromuscular Junction Effects

The neuromuscular junction is known to be susceptible to organophosphate and carbamate chemicals. Several changes have been documented that represent structural and/or biochemical disruption in both the presynaptic motor nerve terminal and the postsynaptic motor end plate region. These alterations are associated with electrophysiological phenomena, and this provides a possible means of monitoring workers who are exposed to the chemical hazard of organophosphate or carbamate pesticide.

Degeneration of the neuromuscular junction was observed in rats by Engel *et. al.* (1973) after 149 days of low dose treatment with prostigmine, an anti-acetylcholinesterase drug used in the treatment of myasthenia gravis. Destruction of postsynaptic folds at the motor end plate resulted in widening of the primary synaptic cleft. An increase in the number of postsynaptic vesicles and an increase in mitochondrial area at the nerve terminal was also described. The presynaptic membrane was either partially or completely covered by a Schwann cell process in the region of the synapse, indicating possible alteration in the normal relationship between nerve and Schwann cells. Degeneration at the neuromuscular junction and altered Schwann cell interaction at the presynaptic membrane would significantly impede communication with the postsynaptic membrane, and could result in increased distal latency as well as other associated symptoms such as muscle weakness.

Electrophysiological changes have been described in animal, clinical, and occupational studies following exposure to cholinesterase inhibitors. These changes involve both positive and negative alterations in miniature end plate potentials (MEPPs), diminishing

amplitude of evoked potentials that follow repeated stimulation, decreased amplitude of evoked potentials after volitional fatigue, and repetitive evoked muscle action potentials after a single stimulus. The observed electrophysiological changes indicate pathology that is not directly related to anticholinesterase effects, but may be due to disruption of the motor nerve terminal itself.

Alterations in MEPPs have been observed in rats administered repeated low level doses of anti-cholinesterase chemicals (Melchers and Van Helden, 1990; Engel *et. al.*, 1973; Fenichel *et. al.*, 1972). Rats treated with prostigmine, a carbamate used in the treatment of myasthenia gravis, showed a 29% decrease in MEPP amplitude with no change in quantum content of the MEPPs (Engel *et. al.*, 1973). Rats injected with the organophosphate paraoxon, however, showed an increase in MEPP amplitude and duration, and a decrease in frequency of MEPPs (Fenichel *et. al.*, 1972). These results indicate that carbamate and organophosphate compounds both influence events at the neuromuscular junction, however, the effect may differ. Indeed, Melchers and Van Helden (1990) demonstrated that the effect exerted by different organophosphates is not identical. They found a decrease in the frequency of MEPPs in rats after chronic administration of DFP, and an increase in MEPP amplitude after chronic treatment with soman.

Jager *et. al.* (1970) summarized earlier research from their laboratory (Roberts and Wilson, 1969) characterizing EMG abnormalities resulting from overtreatment of myasthenia gravis patients with carbamate anticholinesterase drugs (neostigmine and pyridostigmine). Anticholinesterase overtreatment in these patients resulted in low amplitude EMG potentials and repetitive muscle activity after a single stimulus. In a train of four consecutive evoked potentials, the amplitude declined progressively. After ten seconds of voluntary activity in adductor pollicis, the first evoked potential was reduced markedly in amplitude. This depression of the first evoked potential was found to become more pronounced with continued overtreatment, and to decrease following withdrawal of

treatment. Other EMG pathology also disappeared following discontinuation of carbamate treatment, indicating that the observed electrophysiological abnormalities were reversible.

Jager *et. al.* (1970) found that workers exposed to organophosphate and organochlorine pesticides exibited an abnormal EMG similar to that of the overtreated myasthenia gravis patient, even in the absence of any detectable cholinesterase inhibition. A train of four evoked potentials was recorded from the adductor pollicis before and after ten seconds of voluntary activity in the muscle. Stimulation of the ulnar motor nerve in affected workers revealed the following abnormalities: evoked action potentials with a reduced mean amplitude (10 mV ± 1 mV) compared with control subjects (12 mV ± 1 mV), repetitive muscle activity after a single stimulation, and reduced amplitude of the first evoked potential after voluntary activity of the adductor pollicis muscle. The incidence of an abnormal EMG in workers who manufacture and formulate a dimethyl phosphate ester pesticide was about 50% (17 of 36 subjects). Four out of six of these workers who were monitored for EMG changes before and after a five day work-week showed deterioration of EMG parameters during this interval. Six non-pesticide workers with a single acute exposure to organophosphate showed reduced amplitude and repetitive activity a few hours after exposure. These effects recovered significantly within 48 hours. There was no relationship between the EMG change and cholinesterase inhibition, and in most cases of EMG pathology there was no significant cholinesterase depression detected. Whole blood cholinesterase was within the normal range for all but one of the above subjects. The authors concluded that the EMG analysis described is more sensitive to the effects of organophosphate and/or carbamate intoxication than is cholinesterase monitoring .

Wadia *et. al.* (1987) studied several electrophysiological parameters in 2000 patients suffering from acute high level organophosphate intoxication after attempting suicide. They found the amplitude of the compound action potential in abductor digiti minimi (ulnar nerve) and extensor digitorium brevis (lateral popliteal nerve) to be lower in severely affected individuals than in control subjects. A repetitive response to a single stimulus was

seen in 60% of cases tested and in 100% of cases with clinical symptoms. This response disappeared with repeated stimulation. Repetitive activity is deemed characteristic of organophosphate poisoning by these authors and was interpreted to indicate a reversible lesion at the neuromuscular junction. This interpretation was based on findings that a similar electrophysiological response, low amplitude with normal velocity, is observed in Eaton Lambert syndrome and botulism, in both of which lesion of the anterior horn cells is exhibited. Because anterior horn cells are associated with nicotinic cholinergic receptors, there may be damage at this level in addition to the neuromuscular junction in organophosphate exposed individuals. Repetitive stimulation at 30 hertz produced a decreasing response in amplitude between the first and fifth evoked action potential.

Repetitive firing after a single stimulus was found in 29% of workers exposed to a repeated low dose of fenthion (Misra *et. al.*, 1988). The repetitive firing after a single stimulus observed by Jager *et. al.* (1970), Wadia *et. al.* (1987), and Misra *et. al.* (1988) is similar in nature to the post-tetanic repetitive activity (PTR) reported by Standaert (1964). The PTR is deemed to originate in the motor nerve terminal and to result in multiple muscle action potentials as a muscle's refractory period subsides (Standaert, 1964; Olson and Swett, 1971). Olson and Swett (1971) reported finding one to ten action potentials after a single post-tetanic stimulus in homogenous slow twitch muscle (red soleus). These authors reported that only 20% of fast twitch muscle (white medial gastrocnemius) showed PTR due to the longer refractory period of fast twitch muscle and the relatively brief period of repetitive discharge. The latency of both PTR and the repetitive activity reported after organophosphate or carbamate absorption is approximately 15 to 30 msec after the stimulus-evoked action potential.

Drenth *et. al.* (1972) also observed low amplitude action potentials in 40% of agricultural workers tested. Retesting two months later showed a change in status for 25 of 53 workers from normal to abnormal or *vice versa*. No correlation between abnormal EMG and AChE activity was found. The lack of a consistent recovery in a worker with a

low amplitude evoked action potential limits the utility of amplitude measurement in assessing neurological function in a pesticide exposed worker.

Electromyography (EMG) was used by Hussain *et. al.* (1981) as a non-invasive method of monitoring workers for significant organophosphate absorption. Amplitude of the median nerve EMG response and RBC AChE both showed significant depression in agricultural workers compared with controls. The authors also stated that further research was required before EMG could be used as a reliable method of monitoring workers.

Muscle Effects

A progressive myopathy has been observed after chronic paraoxon administration to rats (Wecker *et. al.*, 1978; Fenichel *et. al.*, 1972). Fenichel *et. al.* (1972) found that myopathy started with a focal necrosis at the motor end plate region and progressed to complete muscle fibre necrosis and phagocytosis. This myopathy was directly related to excess acetylcholine at the neuromuscular junction. Denervation and hemicholinium administration prevented the initiation of focal necrosis at the end plate, demonstrating that damage to muscle tissue was a direct result of trophic and/or regulatory neural influences on the muscle fibres. Symptoms of paraoxon intoxication diminished as the production of new lesions slowed on the ninth day of treatment.

Wecker *et. al.* (1978) determined that organophosphate-induced myopathy was specifically related to inhibition of AChE and that the severity of myopathy could be reduced by administering oximes within 10 to 120 minutes of paraoxon exposure. Monitoring AChE activity on a regular basis could therefore prevent myopathology in workers who are exposed to anticholinesterase chemicals.

ANTICHOLINESTERASE EFFECTS

Both organophosphate and carbamate pesticides are deemed to exert a major effect on cholinesterase isozymes through irreversible or reversible inhibition, respectively. This interaction between the pesticide and the cholinesterase isozymes has been utilized to study

and monitor the effects of organophosphate and carbamate exposure. It is important that the biology of cholinesterases is understood if one is to use this enzyme system as a tool in the investigation of pesticide exposure and the resultant effects.

Biology of Cholinesterases

Structure

The biochemical and physiological characterization of the cholinesterase group of enzymes is far from complete; however, much is currently known about the diversity of these isozymes. The two major groups of isozymes are the acylcholine acylhydrolases, also known as pseudo or butyrylcholinesterase (PChE) (EC 3.1.1.8), and acetylcholine acetylhydrolases, the true or acetylcholinesterase (AChE) (EC 3.1.1.7). The two classes are distinguished by their substrate preference and by their differing sensitivity to inhibitors. PChE shows greater affinity for butyrylcholine than acetylcholine and greater sensitivity to inhibition from certain organophosphate compounds (La Du and Lockridge, 1986). Butyrylcholinesterase is the typical smooth muscle, adipocyte, liver and serum form of the enzyme, while acetylcholinesterase is predominantly found in brain, nerve, erythrocytes, and the neuromuscular junction (Brimijoin, 1986; Abiola *et. al.*, 1988).

Butyrylcholinesterase has a molecular weight of 340,000 , of which 24% is carbohydrate. It is composed of four identical subunits of 580 amino acids each, held together through hydrophobic interactions and disulphide bonds. The disulphide bonds are known to be nonessential for catalytic activity or the structure of the tetramer, but are considered to contribute to the overall stability of the molecule (Lockridge *et. al.*, 1979).

The active site of cholinesterases has been described as containing two significant chemical moieties; an anionic site and an esteratic site. The anionic site is due to a glutamic acid residue, which interacts with the nitrogen of choline. The esteratic site contains a serine residue which is acetylated by acetylcholine during enzymatic hydrolysis (Derache,

1977). Nucleophilicity of the serine hydroxyl moiety is increased by hydrogen bonding to a nearby histidine residue.

La Du and Lockridge (1986) used radioactive labelled [^{3}H]-diisopropylflourophosphate to selectively alkylate the serine residue active site, from which it was determined that each subunit contains an identical active site; hence, each PChE molecule contains four serine residue active sites. These authors characterized the amino acid sequence of human PChE and found considerable sequence homology between human PChE and AChE from *Torpedo* fish. This evolutionary homology was interpreted to indicate that PChE plays a significant role in mammalian metabolism; however, the role is still undetermined.

As with PChE, there are several isozymes of AChE that are structurally distinct but surprisingly identical in catalytic activity. The subspecies of AChE are divided into two molecular forms; globular and asymmetric. The globular forms are mono, di, or tetramers of a catalytic subunit (G_1, G_2, and G_4 respectively) which may be attached to a hydrophobic domain. Hydrophobicity is due to the addition of glycophospholipids at the carboxyl terminal of the globular subunit (Schumacher *et. al.*, 1986). The catalytic subunit in *Torpedo* fish consists of 575 amino acids with several sites of N-linked glycosylation and eight cysteines, providing the potential for multiple disulphide linkages. As in PChE, the esteratic active site is characterized by a serine residue at amino acid 200 (Schumacher *et. al.*, 1986). Human erythrocyte AChE (RBC AChE) is a disulphide linked amphipathic dimer of globular 75 kDa subunits (Rosenberry *et. al.*, 1986). The asymmetric forms of AChE contain one, two, or three globular tetramers of catalytic subunits (A_4, A_8, and A_{12} respectively) which are disulphide linked to a collagenous triple helix tail, resulting in molecular weights in the range of 1,000,000 (Rosenberry *et. al.*, 1986).

Both globular and asymmetric forms are synthesized within a cell and secreted to specific extracellular sites where the properties of the glycophospholipid or the collagenous helix determine the specific association between AChE and the plasma or basement membrane (Schumacher *et. al.*, 1986). Asymmetric forms of the molecule tend to be

associated with the basement membrane rather than inserting into the plasma membrane and are typical of AChE in the skeletal neuromuscular junction. The globular AChE tends to be bound to the plasma membrane through the hydrophobic domain as is typical of RBC AChE (Rosenberry *et. al.*, 1986). The AChE found in erythrocyte membrane is therefore structurally different from that found in the neuromuscular junction. Thus it should not be assumed that substrate or inhibitor affinity is identical for both the erythrocyte AChE and the neuromuscular AChE. This possibility should not be overlooked in the interpretation of blood cholinesterase activity measurements for the assessment of anticholinesterase effects.

Function

The role of neuronal AChE is fairly well understood. AChE found in synapses of both the central and peripheral nervous systems, as well as the neuromuscular junction, plays a major role in normal neuroneuronal and neuromuscular transmission. In cholinergic synapses, acetylcholine is released by the presynaptic cell into the synaptic cleft and diffuses across to the postsynaptic membrane where receptor binding initiates the postsynaptic response. AChE hydrolyses acetylcholine to choline and acetate, which do not bind to acetylcholine receptors. This serves to limit the signal duration and intensity, and allows for more rapid reuptake of acetylcholine metabolites by the presynaptic neuron (Trundle and Marcial, 1988).

The role of serum PChE and RBC AChE in normal physiology is not currently understood; however, it has been demonstrated that these forms of the enzyme act as a buffer to protect neuronal AChE from the potentially harmful action of inhibiting agents (Raveh *et. al.*, 1989; Trundle and Marcial, 1988). Recovery from reversible inhibition by carbamate or succinylcholine depends to a large extent on circulating PChE. Succinylcholine is used as a muscle relaxant during some surgical procedures. A genetic variant of PChE which is unable to hydrolyse succinylcholine (suxamethonium) exists in one of every 2500 individuals. Possession of this variant results in an exaggerated

response to succinylcholine with prolonged respiratory and muscular paralysis (Trundle and Marcial, 1988; Silver, 1974). This is due to the inability of the variant PChE to detoxify part of the administered succinylcholine dose, which subsequently results in an elevated effective dose at the synapse. The larger dose of succinylcholine competitively inhibits neuronal and neuromuscular AChE, resulting in paralysis and apnea.

At least four variants of PChE genetic alleles have been characterized: Eu, the normal form; Ea, coding for a dibucaine resistant variant; Ef, coding for a fluoride resistant strain; and Es, a non-expressed silent gene. Normal serum PChE activity may be present in EuEu, EuEa or EaEa variants. Other combinations of the Ea, Ef, and Es alleles may result in decreased serum PChE activity and therefore increased sensitivity to succinylcholine induced apnea (Trundle and Marcial, 1988). It follows that an individual with low serum PChE activity may also be hypersensitive to the effect of anticholinesterase chemicals.

Normal Values of Activity

Ellman *et. al.* (1961) have reported normal RBC AChE activity to be 1.08 +/- 0.16 *f*mol/min/RBC using acetylthiocholine as substrate at 37°C. Using propionylthiocholine as substrate, Voss and Sachsse (1970) have reported normal values for serum PChE and RBC AChE, respectively, to be 0.25 and 0.33 µmoles thiocholine produced/10 µl blood/10 min, respectively. This corresponds to approximately 1.25 µmol/min/ml serum for PChE and 0.6 *f*mol/min/RBC for RBC AChE, if a hematocrit of 0.5 and RBC count of 5.0 TErc/L are assumed. Ratner *et. al.* (1989) have reported somewhat higher values for PChE at 5.0 +/- 0.9 µmol/min/ml; however, RBC AChE is comparable at 36.3 +/- 4.6 µmol/min/g Hb, corresponding to 1.09 *f*mol/min/RBC assuming 150 g/L hemoglobin and 5.0 TErc/L RBC count. The latter authors have also reported no significant difference in activity between men and women. Loosli (1980) noted a large interindividual variation in serum PChE and RBC AChE activity of organophosphate spraymen. Estimates of interindividual variation range from 7.6 to 11.3 percent (Derache, 1977).

Variation in Activity

Seasonal variation in serum PChE activity but not RBC AChE activity has been observed in a study of rural communities in Israel (Ratner *et. al.*, 1989). These authors observed significantly lower PChE in summer compared with winter, which they attributed to increased ingestion of pesticide on fresh fruit and vegetables.

Subnormal cholinesterase activity may be caused by reduced hepatic biosynthesis, changes in hemopoeisis, and the expression of genetic variants of the PChE allele (Roberts, 1980).

Younkin *et. al.* (1986) demonstrated that alteration of axonal transport may influence the quantity of AChE at the synaptic level. Neuronal AChE is synthesized in the soma and transported to the terminal by fast axonal transport. These authors used the variety in molecular form of AChE to investigate the etiology of Alzheimer's disease. They found that there was a significant change in the quantity of both the globular and asymmetric forms of AChE in the Brodmann area 21, of the cerebral cortex in Alzheimer's disease. The authors postulated that a decline in membrane associated G_4, the main cerebral form, was due to the association with abnormal incoming cholinergic axons. A large increase in both A_8 and A_{12} asymmetric forms was due to impairment of fast axonal transport in the incoming cholinergic neurons.

The effect of physical exercise both on cholinesterase activity and on sensitivity to either carbamate or organophosphate intoxication has been investigated primarily in animals and little in humans; however, some interesting results have been reported. Ryhanen and coworkers (1988) found that exercise increased the activity of total blood cholinesterase, serum PChE, and AChE in the brain and diaphragm for as long as 48 hours, but had no significant effect on RBC AChE activity. Pawlowska *et. al.* (1985a) found similar results for a single exercise bout, but a decreased total PChE activity after long term exercise in rats. A postulated mechanism for these changes in serum PChE include increased hepatic production of PChE during exercise. Tissue AChE activity may rise in response to an

increased concentration of acetylcholine which itself results from increased metabolic activity of the tissue in question (Ryhanen *et. al.*, 1988).

Cholinesterase Inhibition

Mechanism

The cholinesterase enzymes are inhibited by both organophosphates and N-methylcarbamates. Organophosphates usually cause irreversible inhibition, while carbamates produce reversible inhibition. Irreversible inhibition by organophosphates is characterized by a two step complexing competitive inhibition. A reversible enzyme-inhibitor complex is formed initially, followed by phosphorylation of the enzyme esteratic site (Gray and Duggleby, 1989). This phosphorylation renders the esteratic site unavailable for acetylation and therefore prevents binding of acetylcholine to the active site.

Organophosphate compounds differ greatly in their potency of inhibition. This has been attributed to a difference in their ability to phosphorylate the esteratic site (Singh, 1985). In some cases, metabolites of an insecticide prove to be more potent inhibitors of cholinesterase than the insecticide itself. Acephate, for example, is deacetylated to form methamidophos, which is 100 times more potent an inhibitor of AChE and PChE. This is due to diminished steric hinderance between the organophosphate and the esteratic enzyme site, producing an increased rate of phosphorylation within the active site itself (Singh, 1985). Similarly, parathion and malathion are less toxic than their respective oxons, paraoxon and malaoxon.

Aging of Inhibited Cholinesterase

Spontaneous reactivation of organophosphate inhibited AChE is a slow process. This allows for another slow process to occur; dealkylation of the bound organophosphate in the esteratic active site leaves a negatively charged residue covalently bound to the active site, which is therefore immune to reactivation (Chambers, 1989; Berends and Posthumus, 1959). This process of slow dealkylation has been called 'aging' of the inhibited AChE and results in a situation where recovery from organophosphate poisoning may only occur

by synthesis of new cholinesterase or utilization of cholinesterase which has avoided contact with the organophosphate (Chambers, 1989). If aging of the phosphorylated site has not taken place, reactivation of the enzyme complex by hydrolysis of the phosphate moiety is possible through the use of hydroxylamine or oxime derivatives (Derache, 1977). This is one method of treatment of acute organophosphate insecticide poisoning. It has been demonstrated that aging of paraoxon-inhibited brain AChE takes four days to reach a level which resists reactivation by oximes (Chambers, 1989).

Recovery

Coye *et. al.* (1987) reported three incidents of multiple worker poisoning in which both serum PChE and RBC AChE activity was serially determined throughout recovery. The various groups differed markedly in the pattern of their recovery. One group of subjects was exposed to mevinphos and phosphamidon which caused an average 66% inhibition of PChE and 32.5% inhibition of RBC AChE, which took 66 days to recover from. Most symptoms disappeared within four weeks, however, headaches, weakness, anorexia and blurred vision persisted in many of the group for up to eighteen weeks. Subjects in group two and three were exposed to mevinphos and diazinon respectively. This resulted in inhibition of 15.6% and 29.4% for PChE, and 5.6% and 27.2% for AChE respectively in each group. All subjects recovered RBC AChE activity within two weeks. Group one subjects were more severely inhibited and took considerably longer to recover. Their exposure to two compounds may have played a role in enhancing the severity of effect.

Milby (1971) reported regeneration of RBC AChE as 1% of control activity per day, while serum PChE recovered at a rate of 25% per week after parathion poisoning.

After organophosphate poisoning in particular, symptoms in humans may subside within one or two days despite a continued reduction in AChE activity (Chambers, 1989). Several cholinergic compensatory mechanisms may be involved in this apparent recovery from overt symptoms, including: 1) a decreased number or sensitivity of postsynaptic

muscarinic receptors, 2) a reduced acetylcholine synthesis by cholineacyltransferase (ChAT), 3) a reduced acetylcholine release per nerve impulse, or 4) a decreased high-affinity choline uptake into the presynaptic terminal (Chambers, 1989).

Prophylactic Treatment

Prophylaxis against organophosphate poisoning has a significant implication for agriculture, industry, and the military.

One approach has been to administer an exogenous AChE which then competes with the endogenous AChE for interaction with absorbed organophosphate. This type of scavenging prophylaxis has been effective in preventing poisoning in animal studies and has been shown to protect endogenous AChE from a dose of organophosphate eight times as large as the LD50 in mice (Raveh *et. al.*, 1989). The animals exhibited only minor symptoms even after an intake of a large dose of organophosphate. Aas *et. al.* (1987) found that blood borne cholinesterase was able to scavenge a chronic low level dose of the organophosphate soman effectively enough to prevent inhibition of AChE in the central nervous system; however, peripheral cholinesterase was significantly affected.

A second approach has been to use reactivators such as oximes to promote dephosphorylation of the esteratic active site, before aging can occur. The administration of a reactivator makes organophosphate inhibition of cholinesterase a rapidly reversible process. Raveh and coworkers (1989) used the reactivator 1,1-trimethylenebis(4-hydroximinomethyl)dibromide (TMB_4) to decrease the sensitivity of mice to a repeated dose of organophosphate. The use of reactivators in addition to exogenous AChE extends the protection afforded by either alone (Raveh *et. al.*, 1989).

A third approach to prophylaxis has been to use reversible inhibition of cholinesterase by a carbamate to protect from the more severe and long-lasting effect of irreversible cholinesterase inhibition by organophosphate absorption (Das Gupta *et. al.*, 1989). This is effective at reducing some anticholinesterase effects of organophosphate intoxication,

however other effects such as depression of the monosynaptic reflex are cholinesterase-independent and are immune to carbamate prophylaxis (Das Gupta *et. al.*, 1989). Wecker *et. al.*, (1978) used pretreatment with a single dose of carbamate to prevent AChE inhibition and muscle fiber necrosis from paraoxon exposure.

Exercise Effects

It has been proposed that an elevated serum cholinesterase activity resulting from exercise may provide protection against intoxication by anticholinesterase chemicals. Ryhanen *et. al.* (1988) tested this hypothesis in rats. One group of rats were exercised at a moderate rate on a treadmill immediately after their injection with an organophosphate compound. A control group was similarly injected with the organophosphate but was not exercised. It was found that during exercise and for up to 30 minutes post exercise serum PChE, brain AChE and diaphragm AChE were not inhibited in the exercise group to the same extent as in control animals. RBC AChE, however, showed greater inhibition during exercise than in non-exercised controls, and there was no change in total blood PChE activity.

McMaster and Finger (1989) found exercise in rats to have a profound effect on behavioural sensitivity to physostigmine, a carbamate compound that acts centrally. It was shown that exercise-trained animals performed better than untrained animals on a multi-component operant task when administered a carbamate compound immediately prior to the task. It was therefore proposed that exercise training may be useful in producing behavioural tolerance to carbamate intoxication in the absence of a prior carbamate dose. This behavioural tolerance is different from the pharmacological tolerance that is typically described in the literature, since no previous dose of carbamate is required to produce the behavioural tolerance.

The exercise history of an individual is also an important factor in assessing the effect of acute exercise on the behavioural response to carbamate exposure (McMaster and

Finger, 1989). The research protocol followed in the above investigation involved an acute exercise bout immediately prior to injection of physostigmine, which was then followed by a multicomponent operant task. Untrained animals showed an increased behavioural sensitivity (negative effect) after acute exercise, as indicated by diminished performance on the operant task. Exercise trained animals also performed worse on the operant task when acute exercise preceded the physostigmine dose, however, the negative effect of acute exercise was much less than in the untrained animals, similarly treated.

Although there is potential for using exercise as a prophylactic measure against the anticholinesterase and behavioural effects of organophosphates and carbamates, much more research is needed to determine the mechanism and degree of such protection. The above research indicates that exercise, alone, does not provide primary protection. The exercise history of an individual and the timing of exercise relative to ensuing organophosphate or carbamate exposure must be controlled. It appears that prior-to-exposure exercise training and post-exposure exercise are both beneficial for minimizing the negative effect of anticholinesterase agents, however, exercise immediately prior to chemical exposure seems to potentiate the response, even in trained individuals (Ryhanen *et. al.*, 1988; McMaster and Finger, 1989). No research has yet addressed the problem of concurrent exercise and anticholinesterase exposure, as would be the likely scenario in tree planting work.

Interactive Effects

The use of multiple pesticides on a single crop is common practice in agriculture. An interactive effect between two or more pesticides has the potential to increase the toxicological risk to a worker who is exposed to a combination of pesticide. Takahashi *et. al.* (1987) demonstrated the potentiating effect of a low dose of either malathion, fenitrothion, or cyanophos, P=S type organophosphate pesticides, on the acute toxicity of 2-*sec*butylphenyl N-methylcarbamate, an N-methylcarbamate pesticide, for mice when the P=S organophosphate was administered in a time period of four hours to one half hour

prior to administration of the carbamate. They defined acute toxicity by the LD50 ratio of pretreated to nontreated animals. Potentiation of acute toxicity was not observed when animals were pretreated with a P=O type organophosphate. These authors also proposed that potentiation was due to inhibition of the metabolic process detoxifying the N-methylcarbamate pesticide. The elevated serum concentration of carbamate in the organophosphate pretreated animal supports this hypothesis. It was also found that pretreatment with SKF 525-A, an inhibitor of mixed function oxidases, produced an elevated serum concentration of carbamate; however, the potentiation of acute toxicity in animals studied was not as great as when they were pretreated with organophosphate. It was concluded that inhibition of detoxifying mechanisms may play a role in potentiating an animal's response to N-methylcarbamate, but it is not the complete reason for the increased toxicity. It should also be noted that there was no difference in carbamate induced anti-acetylcholinesterase effect between the pretreated and untreated animals.

Synergism has been observed in humans exposed to both organophosphate (fenitrothion) and pyrethrinoid (fenvalerate) pesticides (Abiola *et. al.*, 1988). No significant effect on RBC AChE or serum PChE was found when fenitrothion was applied at 500 grams per liter by field workers, however, a deepening depression of both cholinesterases was found when fenitrothion was applied at 250 grams per liter concurrently with fenvalerate at 50 grams per liter under similar field conditions. Pyrethrinoid pesticide alone does not have an effect on cholinesterase activity. These authors also found a synergistic depressive effect on cholinesterase activity in workers who used both azinphosmethyl and deltamethrin in a close time frame.

Symptom Tolerance

Central and/or peripheral symptoms of organophosphate or carbamate intoxication may diminish in severity or disappear completely after chronic or repeated exposure to these chemicals, even though AChE remains significantly inhibited in many tissues. This

phenomenon of induced tolerance to anticholinesterase chemicals is not well understood from a physiological perspective. Aas *et. al.* (1987) have shown that the adaptation of the nervous system to cholinesterase inhibitors depends on the duration and level of exposure as well as the type of inhibitor.

Behavioural tolerance to anticholinesterase chemicals has been studied using biochemical and neurophysiological approaches to elucidate the responsible mechanisms. Van Dongen and Wolthius (1989) exposed rats either to soman or DFP every other day for four weeks. This treatment resulted in progressively decreased cholinesterase activity in various tissues. Central or behavioural effects were assessed by performance in a shuttlebox task administered one hour after organophosphate injection. The toxic effect of the organophosphate chemical on the animal's central nervous system resulted in decreased performance on the shuttlebox task. However, decrement in shuttlebox task performance was significantly alleviated for rats serially injected with DFP, but not for those injected with soman. This demonstrated a developing behavioural tolerance in the DFP exposed rats. It is also important to note that these two inhibitors had different effects with respect to the development of tolerance. Both groups of rats showed normal performance when tested 24 hours after injection, demonstrating short-term behavioural adaptation to the effect of a single dose. The investigators outlined some factors which they proposed played a role in the development of tolerance, such as:

1) a compensatory synthesis of degradative or scavenging enzymes, such as phosphorylphosphatases, carboxylesterases, and cholinesterase isozymes;
2) an undetermined presynaptic effect which would limit the release of acetylcholine and/or cause a reduction in high affinity reuptake of acetylcholine;
3) a synaptic effect such as an increased leak of acetylcholine from the synapse due to neuropathy or myopathy;
4) a postsynaptic effect which would down-regulate muscarinic and nicotinic receptors to reduce their sensitivity and raise the threshold of the postsynaptic membrane;

5) the adoption of behavioural strategies that would allow an individual to manage the effects of low AChE activity; and

6) the development of interactions with other neurotransmitter systems.

Of these possible factors, they found evidence that *de novo* synthesis of AChE in several tissues and down-regulation of muscarinic receptors contributed to a developing tolerance. Van Dongen and Wolthius (1989) were unable to identify any biochemical adaptations that might account for the differing tolerance of humans to soman and DFP.

Several authors have reported a reduction in muscarinic receptor number in direct response to an increase in the synaptic concentration of acetylcholine (Quillfeldt *et. al.*, 1990; Aas *et. al.*, 1987). Quillfeldt *et. al.* (1990) observed a 50% down-regulation of muscarinic receptors in the amygdala within 48 hours of injecting fasciculin, a potent anti-AChE peptide. This reduction in receptor number correlated well with the development of tolerance to further exposure, determined from an avoidance task. Aas *et. al.* (1987) demonstrated that chronic low level inhalation exposure to soman, at a concentration of 0.45 mg/m^2 for 2400 minutes, resulted in a time-dependent reduction in the number of muscarinic receptors in lung and bronchial smooth muscle of rats, even in the absence of peripheral symptoms of intoxication. This down-regulation correlated well with the degree of tissue AChE inhibition due to chronic soman exposure. Brain tissue showed no AChE inhibition and subsequently no change in binding properties. Conversely, an acute high level inhalation exposure, at a concentration of 8.51 mg/m^2 for 45 minutes, resulted in a greater severity of symptoms in rats, but no alteration in receptor binding capacity. This suggests that receptor down-regulation plays a role in the development of long-term tolerance, but not in the response to an acute dose.

The effect of anti-AChE chemicals on cholinergic receptors may involve more than simple alteration of the number of receptors. Katz and Marquis (1989) found a non-competitive modulation of muscarinic receptor binding after paraoxon administration in animals. They isolated three types of muscarinic receptors (M1, M2, M3) and found that

paraoxon exerted an effect on M2 and M3 receptors but not on M1. The affected receptors are associated with presynaptic neurons in the CNS and muscarinic end organs in the periphery. Blocking the binding capacity of M2 and M3 in the CNS reduced negative feedback inhibition on acetylcholine release from the presynaptic neuron, facilitating the behavioural dysfunction observed in chronic low level organophosphate exposure. Alteration in receptor binding was produced with an "ultralow level" of paraoxon exposure, causing no change in AChE activity. The results indicate that loss of homeostasis in the CNS cholinergic system is due to a direct interaction of the organophosphate with the muscarinic receptors in addition to reduced AChE activity. Katz and Marquis (1989) further postulated that any developing cholinergic imbalance would induce other neurotransmitter action (GABA and dopamine) to compensate for cholinergic dysfunction.

Melchers and Van Helden (1990) found a decrease in the frequency of miniature end plate potentials (MEPPs) after chronic administration of DFP, and an increase in MEPP amplitude after chronic injection of soman in rats. These results partially explain the difference in behavioural tolerance between animals exposed to DFP and soman that was observed by Van Dongen and Wolthius (1989), since a decreased frequency of MEPPs would be indicative of an adaptive change in presynaptic acetylcholine release or postsynaptic sensitivity to acetylcholine. An increase in MEPP amplitude resulting from soman exposure is detrimental to any beneficial adaptation which would follow from an increase in postsynaptic receptor sensitivity and/or an increase in the quantal release of acetylcholine. Melchers and Van Helden (1990) also demonstrated an increase in the sensitivity of neuromuscular function, *in vitro*, to application of additional inhibitor to a muscle strip preparation from a soman pretreated animal. The latter observation is also in contrast to the observed tolerance in rats, and is supportive of the notion that a previously exposed individual may be more susceptible to the effect of further inhibitor exposure.

The animals studied by Melchers and Van Helden (1990) and Van Dongen and Wolthius (1989) primarily developed a behavioural pattern of adaptation to

organophosphate intoxication. This is not always the pattern of tolerance development. Dille and Smith (1964) reported that many behavioural symptoms characteristic of central nervous system impairment are associated with chronic exposure to organophosphates in crop dusting pilots. He presented two case studies involving symptoms of anxiety, depression, dizziness, emotional lability, severe irritability, and an inability to perform familiar tasks, all in the absence of peripheral symptoms. Animals which have developed a tolerance to organophosphate intoxication, and therefore do not show peripheral symptoms, may still exhibit neurobehavioural dysfunction such as impaired memory (Costa, 1988). Raffaelle *et. al.* (1987) produced a long-term behavioural central neural impairment in rats exposed to a large single dose of soman. The affected animals showed increased activity in open field observations, a learning deficit in maze performance, and increased reactivity to tactile stimulation. These effects were attributed to neural lesions in various brain regions that were evident upon histological analysis *post mortem* . In light of these observations, the CNS dysfunction reported in crop dusters by Dille and Smith (1964) may have been due to inadvertantly high exposure on a few occasions which produced CNS damage and hence long-term behavioural dysfunction. The lack of peripheral symptoms reported may be due to low dose exposure rather than peripheral tolerance.

An alternate explanation of symptomatic tolerance may be based on enzyme inhibition kinetics. Repeated administration of an inhibitor in a low concentration results in a progressive decline in enzyme activity, however, it becomes increasingly more difficult to inhibit enzyme activity as the absolute activity declines (Zubay, 1983). Chronic administration of organophosphate results in an exponential decline in AChE activity (Van Dongen and Wolthius, 1989). Thus, AChE activity may be depressed to very low levels but does not reach zero activity. If the expression of symptoms was dependent upon the rate of decline of AChE activity rather than its absolute level, symptoms might be expected

to subside due to the declining slope of an exponential decay curve and would then resemble pharmacological tolerance.

II. PESTICIDE EXPOSURE AND ABSORPTION

The processes involved in significant worker absorption of pesticide are outlined in Figure 1. Several factors that influence these processes and are relevant to a tree planter's absorption of pesticide are also listed in Figure 1.

Several aspects of hygienic practice, in theory, could account for the variability in pesticide exposure and absorption, and explain the interindividual variability observed in cholinesterase depression among tree planters. The material, style of clothing and gloves worn or not worn during work have all been implicated in contributing to a worker's dermal absorption of pesticide (McDonald, 1988; Cessna, 1988; Raheel, 1987). Long sleeved shirts and full length pants made of tightly woven fabric and worn beneath water-repellant garments are recommended for optimum safety (Cessna, 1988). Fabric testing has indicated that a 65/35 polyester/cotton blend provides superior protection compared with 100% cotton, polyester, nylon, or acrylic , based on rate of absorption and wicking properties (Raheel, 1987). Gloves made of rubber, neoprene or nitrile provide the greatest degree of protection, while leather or fabric gloves readily absorb pesticides and may actually increase dermal absorption by prolonging contact with the skin and by facilitating concentration of chemicals near the skin (Cessna, 1988).

Oral ingestion of pesticide by a worker may occur due to his eating, drinking or smoking with contaminated hands; by carrying food, drink or cigarettes while working with or near pesticides; and by storing edible supplies near pesticides or pesticide treated areas (Cessna, 1988).

Minimizing both dermal and oral exposure involves careful attention to personal hygiene. Washing hands and face prior to eating, drinking or smoking in the field and showering as soon as possible at the end of the day are recommended (Cessna, 1988). Clothing and gloves should be washed or changed daily to minimize contact with pesticide absorbed by the fabric or on the fabric surface (Cessna, 1988).

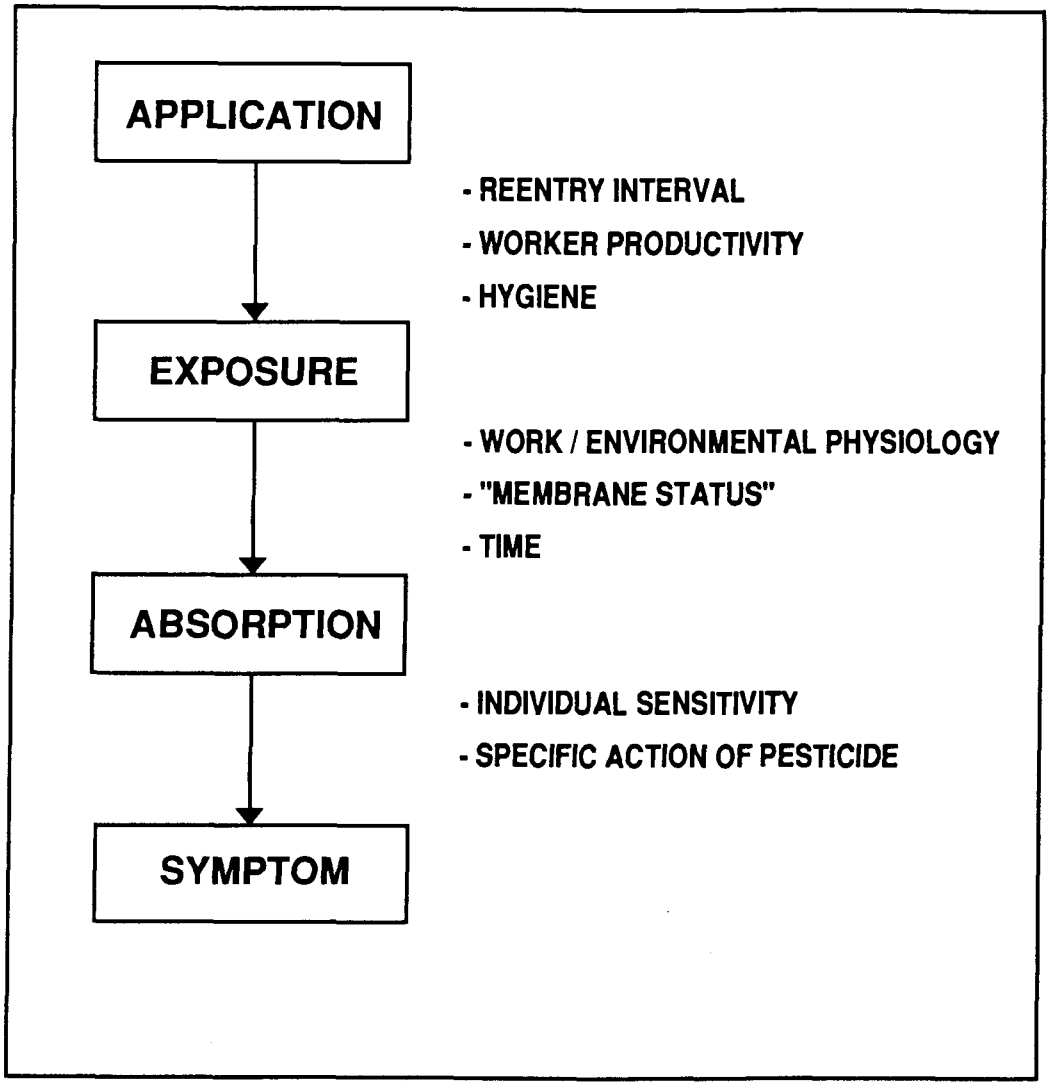

Figure 1 Processes and factors that are involved in a worker's significant absorption of pesticide.

FACTORS INFLUENCING PESTICIDE ABSORPTION

Pesticides may be absorbed dermally, or be inhaled or ingested through pulmonary or alimentary routes respectively. Organophosphates and carbamates are readily absorbed through skin, lung and gastrointestinal membranes (Ngatia and Mgeni, 1980). The relative importance of these routes is specific to the pesticide formulation being used in the individual's specific work practice, the physical severity of work, and the personal hygiene of the worker (Speight, 1980; Spear *et. al.*, 1975).

Pesticide Formulation

Powder formulations enhance both inhalation and ingestion exposure due to the ease at which dust or particulate clouds are produced during handling of treated plants or the pesticide itself. The size of particle in the powder is significant in determining risk of exposure, since very small particles take a considerable time to settle once disturbed. Particulates of 10 micron diameter settle 3 feet in 100 seconds, while a 1 micron particle takes almost 3 hours to settle the same distance (Speight, 1980). Particulate size is also relevant to penetration of pesticide through a worker's clothing. Smaller particles may penetrate through protective clothing right to a worker's skin (Spear *et. al.*, 1975).

A liquid formulation spreads readily across the skin accelerating dermal absorption (Speight, 1980). Prinsen and Van Sittert (1980) observed inhalation exposure to be only one percent of dermal exposure in workers applying a liquid pyrethroid. The spreading characteristic of a liquid preparation is more important in the application or the mixing of a pesticide and is of less significance to a tree planter or harvester since the formulation will have dried substantially or have been absorbed into the plant prior to handling. At this latter stage of contact, the primary concern has to be for dislodgeable surface residue on the plant.

Skin penetration of a pesticide depends on its water solubility more than oil solubility (Dedek, 1980). Both penetration and systemic distribution are greater for hydrophilic

pesticides than hydrophobic pesticides (Dedek, 1980). Furthermore, cholinesterases tend to be found in a hydrophilic environment, and are therefore more susceptible to inhibition by hydrophilic compounds (Dedek, 1980). Lipid soluble pesticides accumulate in adipose tissue and are less available to immediately act physiologically. However, acute storage of these pesticides in adipose tissue will enable an accumulation of a large dose which may then release slowly during an extended period.

Specific Work Practice

Lamb (1980) found that a sixty minute sample taken from the glove or arm of a citrus picker showed a concentration of azinphos methyl 300 times that on the fruit or leaves. Thus dermal and inhalation exposure to dislodgeable pesticides are enhanced through repetitive handling of treated plants. Lamb (1980) also found that the level of pesticide exposure to harvesters was directly related to the rate at which the pesticide had been previously applied to the crop. A high application rate deposits a large concentration of pesticide on foliage, which increases the foliar residue and the related exposure hazard to the worker who subsequently handles the plant.

With few exceptions, incidents involving multiple symptoms due to occupational pesticide intoxication have been attributed to working with foliage that is at least chest high (Spear *et. al.*, 1975). Hence the type of crop and its size are relevant factors in determining potential for foliar residue exposure to workers. Tree planters rarely work with chest high seedlings, but tree seedlings are carried in bags worn around the waist and can rise to shoulder height.

Work Physiology

Ventilatory rate and sweating rate are important variables influencing an individual's pesticide absorption (Spear *et. al.*, 1975). However, the degree of influence is difficult to quantify since both respiratory rate and sweating are dependent on work rate, environmental conditions, clothing, and individual idiosyncrasies. Richter *et. al.* (1980)

support the view that environmental conditions and work physiology contribute to pesticide absorption. In pilots studied by Richter *et. al.* (1980), employed for aerial spraying of organophosphate pesticide in hot environmental conditions, heat stress seemed to potentiate organophosphate absorption due to increased peripheral vasodilation and sweating. Increased organophosphate absorption acts to accentuate the sweating response which in turn increases pesticide absorption further.

Hygiene

Occupational and personal hygiene play an important role in protecting an individual from pesticide exposure. These attributes have specific importance for protection against dermal, gastrointestinal and respiratory absorption of pesticide residue. For a discussion of specific practices, refer to the chapter "Safety Measures/ Occupational Hygiene".

EXPOSURE ASSESSMENT

Reentry Models

Milby *et. al.* (1964) have introduced the reentry concept which proposes a period, unique to each pesticide, between the time of pesticide application and the time that the level of pesticide contamination has decayed to a safe level for worker exposure. A mathematical model, proposed by Serat (1973), to predict a safe reentry interval is based on the decay kinetics of dislodgeable foliar residue and the rate of change of blood cholinesterase activity in an exposed worker. The model does not account for a variety of other intervening factors which influence an individual's toxicological response to a given concentration of dislodgeable foliar residue. A conceptual model of factors affecting residue intoxication was introduced by Spear *et. al.* (1975) including environmental factors, agricultural work practices, work physiology, personal hygiene, and human toxicological processes. This model also include several other probable intervening factors, however no empirical experiments were performed to validate the model. Incorporation of any significant representation of intervening factors into a quantitative dose-response model has proven to

be a difficult task, although several researchers have tried (Gunther *et. al.*, 1977; Popendorf and Leffingwell, 1982). Establishing a quantitative dose-response model is confounded by the inaccuracy of using animals to represent human dose-response sensitivity. Aside from the obvious difficulty in mimicing human agricultural work practice and hygienic practice in an animal experiment, there is a significant difference between human and animal skin, regional differences in dermal absorption across the body surface, and differences in metabolic detoxification (Knaak, 1980).

Popendorf and Leffingwell (1982) proposed the Unified Field Model to address the reentry problem in a predictive, quantitative manner. This model contains a series of four mathematical equations inter-relating: 1) quantity and type of applied pesticide, 2) pesticide residue at any time after application, 3) worker dermal dose, 4) absorbed dose, and 5) fraction of RBC AChE inhibited. From this model, it is possible to determine an acceptable reentry interval based on knowledge of the pesticide, pesticide application rate, pesticide decay kinetics, crop properties, pesticide dermal absorption properties, and the dermal LD50 for the pesticide. The model may be used for multiple organophosphate exposures, however it is assumed that any effect is additive and not synergistic. The model is conceptually powerful for predicting a reentry interval for a well defined field scenario in which pesticide residue, decay kinetics, work practices and worker hygiene have been carefully characterised to quantify the coefficients required by the mathematics of the model. However, these parameters have been quantified for very few work sites. The principal limitation of using a quantitative reentry model is the lack of a consistent foliar residue decay rate (Popendorf and Leffingwell, 1982). Occupations such as tree planting that span several climatic seasons will experience a seasonal change in clothing, decay kinetics, and dermal absorption. Such seasonal variability may result in a significant difference in dose-response from season to season, warranting the establishment of seasonal reentry intervals.

The Unified Field Model also has theoretical power in assessing the potential for a cumulative effect due to chronic pesticide exposure (Popendorf, 1985). The model does not include any modeling of degradation or catabolism of the absorbed dose however, and is therefore of limited use when considering a work cycle which involves both work days and rest days. During a five day work week an individual may not experience a significant pesticide effect, but if the rest interval between work weeks does not allow for complete recovery from an accumulating, but not yet significant, detrimental exposure, a significant effect may be experienced after a period of several more work weeks. Conversely, the calculated effect may be greatly over-estimated if the exposure is simply assessed as an accumulated effect of total work days with no regard for rest days.

Knaak (1980) has suggested evaluating the reentry interval by monitoring cholinesterase activity inhibition in field workers while varying reentry time after a controlled pesticide application. This would begin with a long reentry interval and subsequent trials would have progressively shorter reentry intervals until a physiological response is detected. This approach requires considerable expense and time, involves ethical problems due to the use of human subjects, provides reentry times valid for limited environmental and application conditions, and is valid for the single application of a specific pesticide. The interactive effect of multiple different pesticides during an exposure is not accounted for in this latter method of assigning a reentry interval.

A similar approach to the Unified Field Model of determining an adequate reentry interval is to directly determine the concentration of foliar residue that corresponds to a no-effect-level of pesticide at the time of reentry. This concentration of foliar pesticide residue must be determined while sequentially decreasing the reentry interval for a given crop until a pesticide-induced effect is observed in the workers. This identifies the minimum foliar concentration of pesticide that will result in a pesticide-induced effect, and therefore also identifies the maximum no-effect-level of foliar pesticide residue. This safe level of pesticide residue on foliage can then be used in the future as an indicator for safe reentry.

The error in reentry determination resulting from a variation in environmental factors is reduced by measurement of foliar residue, since this method focuses on actual exposure dose rather than relying solely on reentry time. Spear *et. al.* (1975) have considered that weather related variables may alter the composition of foliar residue. This presents a significant difficulty in using foliar residue analysis to determine reentry time. For example, a parathion residue may have decayed to a non-significant level, but a concomitant increase in paraoxon concentration occurs from this degradation. Measurement of parathion residue alone would indicate safe reentry, while the degradation product paraoxon actually increases the toxicological hazard. Thus it is imperative that a 'safe foliar residue' determination also accounts for toxic degradation products in addition to the applied pesticide. Knaak (1980) defines safe foliar residue as a level which does not produce any or all of the following conditions in workers: 1) cholinesterase activity depression, 2) change in behaviour, 3) reduced sperm count, 4) decreased functional lung tissue, 5) developmental abnormalities, and 6) ataxia. The above definition is also used by the California Department of Food and Agriculture in establishing reentry regulations (Knaak, 1980).

Cholinesterase Monitoring

Theory

Free organophosphate is rapidly metabolized within human tissue and is reduced to an insignificant level within a matter of days. This, combined with the nature of irreversible enzyme inhibition, makes measurement of the free organophosphate level in blood or urine a poor method of exposure assessment (Derache, 1977). For this reason, methods which assess an induced effect have been developed to assess an organophosphate and/or carbamate exposure that would be detrimental to a worker's health.

Measurement of blood cholinesterase activity has been widely used to assess detrimental exposure to anticholinesterase pesticides (Magnotti *et. al.*, 1988; Trundle and

Marcial, 1988; Coye *et. al.*, 1987; Ryhanen and Hanninen, 1987; Copplestone, 1980; Vandekar, 1980). The Scientific Committee on Pesticides of the International Association on Occupational Health stated that "the best practical method for determining organophosphate exposure is measurement of cholinesterase activity" (Tordoir and van Heemstra, 1980). Inhibition of AChE is considered a measure of health risk due to exposure to organophosphate or carbamate pesticides. Prevention of AChE inhibition is deemed important in preventing effects of chronic low level exposure (David and Fairchild, 1980). Permissable levels of organophosphate and carbamate exposure are based on observation of their so-called 'no-effect-levels' on AChE activity (David and Fairchild, 1980).

N-methylcarbamates are reversible inhibitors of cholinesterases, which presents a problem in the assay of their effect because of spontaneous decarbamylation of the inhibited enzyme. Spontaneous enzyme reactivation is of particular concern when a dilution sequence is required in the assay of the associated cholinesterase inhibition. The rate of this reactivation is temperature dependent with a half life of 19, 20, and 38 minutes at 38, 30, and 25 degrees centigrade respectively (Voss and Sachsse, 1970). This must be considered when using incubation or kinetic methods to assay cholinesterase activity.

Care must be exercised in assigning a clinically allowable decrease in cholinesterase activity since using group mean data to determine if a decline is significant may mask an exaggerated response of a susceptible individual, thus putting that individual at risk. It is desirable therefore to monitor individuals rather than groups to determine a range of allowable inhibition of cholinesterase activity or to determine the safety of a worksite (Serat, 1973). Because the normal range of PChE and AChE varies widely between individuals, it is possible for an individual to have significantly inhibited cholinesterase activity after pesticide exposure and still show a level within the normal population range (Coye *et. al.*, 1987; Midtling *et. al.*, 1985; Wolfsie and Winter, 1952; Callaway *et. al.*, 1951). Coye *et. al.* (1987) have presented a theoretical discussion of the problem of false

negative diagnosis based on this fact. If RBC AChE is 25% inhibited, 46 to 90% of the population would still have activity above the lower limit of the normal range. For serum PChE, 92 to 96% of the population would still be within normal range if there was 25% inhibition of the normal value, while 32 to 80% would be within normal range if the normal value were 50% inhibited. Subnormal enzyme activity in an individual may also be caused by reduced hepatic biosynthesis, a change in hemopoietic activity, or the possession of a genetic variant of the cholinesterase allele (Roberts, 1980). The relatively large interindividual variation in activity necessitates a comparison with pre-exposure baseline values during any clinical determination of pesticide-induced cholinesterase inhibition (Trundle and Marcial, 1988). It is essential therefore that a worker's serum PChE and RBC AChE activity be accurately determined prior to his/her exposure for further monitoring purposes. This requires at least three samples of blood cholinesterase taken twenty-four hours apart, the activity of which may be averaged to obtain a normal baseline value for the individual (Trundle and Marcial, 1988). Subsequent post-exposure samples for a given worker may then be compared with a pre-exposure baseline activity. A prework blood sample must be obtained if pesticide exposure has occurred between the day of baseline activity determination and the day of testing (Tordoir and van Heemstra, 1980).

Callaway *et. al.* (1951) provided ground-breaking research in the use of cholinesterase activity as an indicator of organophosphate exposure. This group demonstrated that a significant ($p \leq 0.05$) inhibition of serum PChE and RBC AChE was 20% and 15% respectively when compared with a single pre-exposure measure of enzyme activity. Sixteen percent and 13% inhibition respectively was significant when baseline activity was more carefully determined as the average of three pre-exposure values. Present guidelines propose that a depression of RBC AChE activity 23% below a single pre-exposure value, or 17% below a multiple pre-exposure average value indicates a significant exposure to pesticide. Variation in serum PChE activity is greater than RBC AChE activity; therefore, a depression of 30% below a pre-exposure value is deemed significant (Derache, 1977). The

World Health Organization (1975) guidelines suggest that an AChE inhibition of 30% in workers exposed to pesticide warrants retesting and investigation of the worksite causing the inhibition. Inhibition of 50% warrants removal of an individual from the offending worksite until cholinesterase activity returns to a pre-exposure level, and a thorough investigation of the source of contamination

It has been demonstrated, however, that mild to moderate organophosphate poisoning may produce cholinergic symptoms and discomfort with much less than a 50% depression of PChE (Coye *et. al.*, 1987). Aas *et. al.* (1987) observed minimal peripheral symptoms in rats that were chronically exposed to low level organophosphate (soman), even with 70% inhibition of cholinesterase activity. These authors observed an increase in the severity of peripheral symptoms after an acute high level exposure which resulted in less cholinesterase depression than a chronic low level exposure. Similarly, Sumerford *et. al.* (1953) reported both individuals with severely reduced cholinesterase activity and minimal symptoms, and those with cholinesterase activity in the normal range who showed obvious symptoms. The appearance of peripheral symptoms is apparently more dependent on the rate of decline in PChE activity than on the magnitude of the absolute decline (Aas *et. al.*, 1987; Coye *et. al.*, 1987; Sumerford *et. al.*, 1953).

It has been noted that low level inhibition may be deemed insignificant statistically, however a cumulative biological effect may be occurring such as incipient pharmacological tolerance to the toxic effects of a pesticide (Callaway *et. al.*, 1951). This adaptation becomes significant in a practical sense because it suppresses warning signs and symptoms and allows chronic low level and larger doses to pass unnoticed by an individual. This may lead to physiological damage that may otherwise be prevented by earlier intervention.

Pre- versus post-exposure comparison is the preferred method for occupational monitoring, however, in clinical testing and some occupations this is not practically possible. Coye *et. al.* (1987) have successfully used serial post-exposure cholinesterase determinations to assess organophosphate poisoning when pre-exposure values are

unavailable. They determined that three consecutive examinations three to five days apart showing a recovery through a progressive increase in activity, or two examinations showing either a 20% or greater increase in serum PChE or a 10% or greater increase in RBC AChE activity would be sufficient to confirm cholinesterase inhibition clinically. They suggested further that a complete clinical recovery should be determined by a return of RBC AChE to a pre-exposure or plateau level. Hodgeson and Parkinson (1985) successfully used this method to identify organophosphate intoxication in office workers, even though no baseline enzyme activity data were available and all measures were within the normal range.

Serum PChE activity is more sensitive to organophosphorus and carbamate inhibition than AChE; however, RBC AChE activity is believed to reflect neuronal AChE activity more accurately (Trundle and Marcial, 1988; Duncan *et. al.*, 1986; Roberts, 1980). Once these enzymes are inhibited by organophosphorus compounds, the primary route of return to normal activity is through biosynthesis of new enzyme, particularly for inhibitors which readily age. This may occur relatively rapidly for PChE which is produced by liver; however, erythrocytes do not retain their biosynthetic capability in the circulation. Thus RBC AChE activity cannot recover until there has been adequate turnover of erythrocytes, during which inhibited cells are removed from circulation and newly released erythrocytes provide a new source of uninhibited enzyme (Trundle and Marcial, 1988). Erythrocytes remain in circulation for an average of 120 days (Ganong, 1983). It follows from these arguments that:

1) Serum PChE activity will recover in a shorter period of time than RBC AChE activity and is therefore more sensitive than RBC AChE when using serial assessment of cholinesterase activity recovery to diagnose inhibition
2) RBC AChE is a more appropriate measure when the first sample is obtained several days after exposure, since serum PChE activity recovers rapidly

3) RBC AChE is more likely than serum PChE to show a significant staged decline in activity during serial measurement to assess the cumulative effect of chronic anticholinesterase exposure (Coye *et. al.*, 1987).

Blood cholinesterase activity is a sensitive measure of organophosphate or carbamate exposure, however, one must be cautious in extrapolating from blood cholinesterase activity to neuronal AChE activity. Pesticides have a different affinity for different enzyme systems, as well as exibiting an unequal distribution between tissues (Roberts, 1980). Hence, a large cholinesterase inhibitor effect in the blood does not necessarily reflect a large effect in nervous tissue.

Assessment of PChE inhibition is significant not only in determining the extent of current exposure to pesticides, but also in determining a person's sensitivity to subsequent exposure. Individuals with an already decreased activity of serum PChE or RBC AChE are more susceptible to poisoning by further organophosphate or carbamate exposure, since blood borne PChE may be regarded as a buffering system for removal of anticholinesterase chemicals from circulation prior to their influencing neuronal AChE (Coye *et. al.*, 1987; Midtling *et. al.*, 1985). A reduction in this buffering capacity leaves the neuronal AChE more accessible to further insult by anticholinesterase chemicals.

Assay Methods for Cholinesterase Activity

Several methods have been developed to determine cholinesterase activity utilizing acidometric, gasometric, radiometric, tintometric and photometric detection schemes. The most sensitive and practical method is the photometric scheme, which is based on a variation of the method of Ellman *et. al.* (1961). Ngatia and Mgeni (1980) were able to demonstrate significant inhibition of serum PChE using the Ellman method, even though whole blood cholinesterase measured using the tintometric method was unaffected.

Ellman's method uses an acylthiocholine substrate and 5,5'-dithiobis(2-nitrobenzoate) (DTNB) as the chromogenic indicator in a coupled reaction as follows:

$$\text{acylthiocholine} \xrightarrow{\text{AChE}} \text{thiocholine} + \text{acyl acid}$$

$$\text{thiocholine} + \text{DTNB} \longrightarrow \text{yellow product.}$$

The time course of liberation of yellow product is followed as the change in absorbance at a wavelength of 412 nm (ΔA412) and is directly proportional to the rate of substrate hydrolysis. Enzymatic hydrolysis of the acylthiocholine substrate is the rate limiting step. The use of a blank assay containing DTNB but no substrate accounts for nonenzymatic production of thiols by the tissue being assayed, and allows for sensitive determination of cholinesterase activity. A similar method is employed by some clinical laboratories where the chromogen is 2,6-dichlorophenolindophenol, a blue dye which reacts with the free sulfhydryl to become colourless (Trundle and Marcial, 1988). Selection of the acylcholine substrate depends on the isozyme of cholinesterase to be measured. AChE is most sensitive to acetylthiocholine, PChE responds fastest to butyrylcholine. This method is easily adapted to measure cholinesterase activity in whole blood, serum, or other tissues.

Direct measurement of RBC AChE is a more difficult problem due to the presence of serum PChE in blood. Two methods may be employed to solve this problem. The erythrocytes may be separated from serum using centrifugation and subsequently washed to remove traces of serum. Alternatively, quinidine sulphate may be used to inhibit serum PChE selectively. These methods are not optimal since cell washing is a skilled, laborious process, and the accompanying selectivity of quinidine is not absolute. Quinidine may inhibit some AChE or fail to inhibit all of the PChE. For these reasons, indirect determination of RBC AChE is preferable under most conditions. Voss and Sachsse (1970) used the method of Ellman *et. al.* (1961) to determine the activity of both total blood cholinesterase activity and serum PChE activity. The difference between the two measures represents the activity due to RBC AChE. This method requires only ten minutes incubation during enzymatic hydrolysis and is therefore suitable for assessment of carbamate inhibition of cholinesterase, which is susceptible to spontaneous reactivation.

Several investigators have developed efficient methods of measuring blood cholinesterase activity in the field (Magnotti *et. al.*, 1988; Ryhanen and Hanninen, 1987) as well as using isolated acetylcholinesterase to determine the concentration of low level organophosphate residues (Hammond and Forster, 1989).

Occupational Monitoring Studies

There is an enormous quantity of literature pertaining to monitoring cholinesterase activity in the assessment of occupational exposure to anticholinesterase chemicals. Duncan *et. al.* (1986) have summarized the findings of a number of studies as the percentage of exposed workers with PChE activity below normal. These authors reported a range of results, 7.9% to 99.2% of workers demonstrating subnormal PChE activity. The occupational groups with the largest percentage of workers below normal activity were grape pickers (99%), migrant field workers (82.9%), pesticide factory workers (70.2%), and pesticide applicators (39.4 to 47.2%).

Wicker *et. al.* (1979) monitored RBC AChE and serum PChE activity for eight consecutive seasons in workers who monitor cotton growth and found a significant decline in group mean RBC AChE activity in four of the eight seasons. These authors chose not to measure pesticide residues since it was deemed impractical to monitor a large number of individuals who worked in a multiple number of different cotton fields. They reported that affected individuals complained of "not feeling well" the evening after being highly exposed, but demonstrated no overt symptoms of organophosphate intoxication, even when RBC AChE activity was 50% depressed. Evidence of a cumulative effect on RBC AChE activity was observed during the last four weeks of a ten week monitoring program. Serum PChE was significantly depressed through the fifth and sixth weeks, while RBC AChE was significantly depressed only during weeks seven through ten.

Loosli (1980) used serum PChE and RBC AChE measurement to assess the safety and hygienic practices of eight organophosphorus spraymen under both field and supervised

conditions. Although one individual exhibited a 40 percent decline in serum PChE without a significant change in RBC AChE, it was determined that adequate precautions were generally taken by the individuals involved. It was also noted, however, that these subjects showed very large interindividual variation in both PChE and RBC AChE, while intraindividual variation was minimal and correlated well with incidents of exposure.

Ngatia and Mgeni (1980) monitored serum and whole blood cholinesterase in employees of the Tropical Pesticides Research Institute in Arusha, Tanzania. It was found that employees of the agricultural entomology section who used a mixture of fenthion, carbendazim, and endosulfan showed a significant ($p \leq 0.025$) decrease in serum PChE but not whole blood cholinesterase. Furthermore, employees who were not involved in pesticide use showed no significant change in either parameter.

Burgess and Roberts (1980) carried out a two year longitudinal monitoring study of organophosphorus workers involved in manufacturing and formulation. Serum PChE dropped to 50 percent of a pre exposure value and maintained this low level until thirty days after cessation of pesticide production. Red cell AChE, however, increased after the initial exposure and declined even further on subsequent exposure. This surprising trend emphasizes the need for serial measures of AChE activity throughout a period of pesticide exposure rather than a single pre and post exposure sample if adequate monitoring of any toxic effect is to be made. The above investigation noted that serum levels of PChE activity more closely reflected a change in work protocol than did RBC AChE activity. In observing serial trends, Burgess and Roberts (1980) found it useful to express data as a CUSUM value, where the CUSUM value was the cumulative sum of the difference between monthly means of exposed versus control subjects. Subsequent improvement in hygienic practice brought serum PChE activity back to within 75 percent of the control value.

Lamb (1980) monitored PChE and RBC AChE to determine the reentry time for citrus pickers. A significant decrease in both parameters was noted under two conditions. The

first condition resulted from use of a liquid spray mixed from a wettable powder azinphos methyl. Fruit was picked seven days after and blood was drawn from fruit pickers for analysis of anticholinesterase activity on day ten. Serum PChE showed a larger decline than RBC AChE. The second condition involved spraying of azinphos methyl from a liquid concentrate; harvesting commenced seven days later and blood was drawn from the pickers fourteen days later for assay. In the second condition, RBC AChE showed a larger decline than the serum PChE activity. The difference in the relative response to the two conditions may have been due to a compensatory liver mechanism which increased serum PChE activity in the second condition.

Abiola *et. al.* (1988) observed a developing inhibition of RBC AChE and serum PChE in Senegalese crop protection workers who were applying a combination of fenitrothion and fenvalerate. Inhibition reached 77% of baseline RBC AChE activity and 25% of baseline serum PChE activity after two weeks of daily exposure. After thirty days of recovery, serum PChE activity was still 15% depressed. No significant cholinesterase effect was noted in workers applying fenitrothion alone.

III. SAFETY MEASURES/OCCUPATIONAL HYGEINE

APPROACHES TO SAFETY

Occupational safety is the responsibility of both the employer and the employee. While ultimately it is the worker's responsibility to work safely and alertly with respect to him/herself and others, it is the employer's duty to ensure both that a safe working environment is available and that the workers are motivated to develop safe work habits. This requires confirmation that safety measures are adequate for the job in question. For workers using organophosphate pesticide, this should involve 1) a regular medical examination to monitor subclinical evidence of exposure, 2) either a regular cholinesterase activity screening or quantification of a pesticide and its metabolites in blood or urine (Roberts, 1980), and 3) the maintenance of a reentry log that contains the pesticide application history and the reentry interval for each crop or field, easily accessible to a worker who will be exposed to the pesticide.

Popendorf and Leffingwell (1982) outlined a more holistic approach which included multiple levels at which safety measures may be implemented. These include engineering, administration, work force management, worker monitoring, and personal protection. From an engineering point of view, mechanization of any pesticide handling job to minimize contact between the worker and the pesticide is desirable. This is effective at the formulation, mixing and sometimes application stages of pesticide use, but is not practical or possible for many field jobs such as tree planting or food harvesting. Any administrative control which is based solely on foliar residue monitoring to establish safe reentry is expensive, limited by poor characterization of residue decay kinetics, and not easily applied to situations where there is exposure of a worker to multiple pesticides. Management of the work force to limit frequency and duration of exposure is an effective method of preventing a chronic exposure problem, but does not protect against inadvertent acute poisoning and may be unpopular among workers whose salaries are based on a piece-

work structure. Monitoring a worker's blood cholinesterase activity is not always feasible as a long term monitoring process due to the logistic and financial constraints in monitoring a large work force, as well as the understandable hesitancy of most workers in providing regular blood samples for analysis. Protective clothing theoretically can provide 100% protection against pesticide exposure, however, adequate personal protection is often expensive, limits mobility and/or dexterity, and causes thermoregulatory problems in an occupation that is physically demanding or located in a warm climate.

PROTECTIVE CLOTHING

The use of protective clothing is appropriate when handling pesticide or pesticide treated materials, and the importance of minimizing dermal contact has been demonstrated in several investigations.

Lamb (1980) observed that the level of azinphos methyl measured on the gloves and arms of citrus harvesters was as high as 300 times the level on fruit and leaves. On the basis of these measures, he suggested that even minimal protection from dermal exposure would significantly decrease the quantity of pesticide absorbed.

Hussain *et. al.* (1990) used patch measurement of pesticide residue on skin, clothing and in the breathing zone of grain farmers who were applying pesticide. The vast majority of exposure (99%) was found to be dermal, 87% of the total dose coming from hand and wrist exposure. Work clothes with disposable Tyvek coveralls were found to afford 60% protection by comparison of residues on clothing and skin. The large percentage of pesticide dose found on the hands and wrist was testimony to the necessity of wearing good gloves.

Wicker *et. al.* (1979) found that cholinesterase depression among cotton field workers correlated well with poor hygiene. Individuals with little regard for the reentry interval and those who failed to change clothing daily were more likely to experience serum PChE

depression. Exposure susceptibility was also increased when clothing became wet, illustrating the need for adequate water proof clothing when working under wet conditions.

Use of gloves, long sleeved shirts, daily change of work clothing, and post-exposure bathing have been recommended to minimize pesticide dose (B.C. Research, 1988; Cessna, 1988). Milby (1971) emphasized the need for conscientious attention to hygiene by individuals exposed to pesticides. He noted that wearing gloves contaminated by pesticide on the inner surface accentuates absorption of the pesticide by the worker. Regular washing or replacement of gloves, along with selection of a work glove with a cuff that either tucked under the sleeve or sealed around the wrist minimized contamination of the inner surface of the glove.

B.C. Research (1988) found that the use of "chainsaw" gloves with an inner glove liner or the use of "dishwashing" gloves significantly reduced dermal hand exposure to pesticide among tree planters. It was also found that cotton glove liners tended to concentrate pesticide residue when the liners became wet, therefore increasing dermal exposure. These authors recommended the use of an impermeable glove liner inside the chainsaw glove, which is an open-weave nylon glove favoured by a large number of tree planters.

Penetration of pesticide through protective clothing depends on the pesticide, its formulation, the composition and thickness of clothing material, and the duration of exposure (Speight, 1980; Dedek, 1980). Exposure time may be reduced through conscientious, prompt removal and washing protective clothing free of contaminant as soon as possible after exposure (Speight, 1980). Penetration rate through protective clothing has been found directly dependent on clothing permeability, and inversely dependent on pesticide water solubility (Dedek, 1980). According to Dedek (1980) the greatest protection against organophosphate absorption is afforded by a butyl rubber vulcanisate, which is 100 times less penetrable than PVC of similar thickness.

IV. TREE PLANTING

ANECDOTAL TESTIMONY FOR A PROBLEM

Spear and coworkers (1975) point out that most severe, occupationally-related, pesticide poisoning occurs among workers who mix, load or apply concentrated chemicals. However, poisoning which follows exposure to foliar residues presents a significant problem which must be addressed for the following reasons:

1) there are a very large number of workers exposed to foliar pesticide residues;
2) these workers tend to be part of a transient, seasonal work force that is therefore very difficult to monitor with traditional occupational hygiene methods;
3) workers are seldom aware of the presence of a pesticide residue on the foliage or on their person, or of its significance;
4) exposure to such a residue does not often lead to intoxication.

These statements appear directly applicable to the tree planting work force in British Columbia.

Several isolated incidents have been unofficially reported in which tree planters present severe symptoms of organophosphate intoxication under circumstances which suggest possible pesticide exposure. In 1981, the personnel of a tree planting camp near Terrace, B.C. reported severe symptoms in the half of the work force that planted, and none in the half that refused to handle the tree seedlings due to an apparently high level of pesticide residue (Vancouver Sun, 1981). In August 1988 near Fort St. John, B.C., several treeplanters exhibited anticholinesterase symptoms after planting seedlings which had recently been treated (Screef, 1989). Unfortunately, in neither case was the pesticide or blood cholinesterase concentration determined, however, water-borne giardiasis was ruled out in Fort St. John. This type of information, while far from conclusive, does indicate a potential occupational hazard for treeplanters from pesticide exposure.

PESTICIDE USE AND NOTIFICATION

Tree seedlings grown in British Columbia provincial nurseries may be treated with the following pesticides as required to minimize damage from a variety of pests (G. Shrimpton, Nursery Pest Management Officer, B.C. Forest Service; personal communication, 1991):

1. Herbicides: A.W.K. and Simazine
2. Fungicides: Rovral, Benlate, Captan, and Bravo
3. Insecticides: Diazinon, Sevin, Belmark, Ambush, Cymbush, Orthene, and Safer's soap.

The policy of B.C. nurseries is to use pesticides only when non-chemical methods of pest control have been ineffective, and in compliance with all notification, registration, environmental, and regulatory agency requirements. Pesticide use in Ministry nurseries declined by 80% between 1986 and 1991, and 30% of tree stock produced in 1991 was pesticide-free (G. Shrimpton, personal communication 1991).

In 1981 a program was implemented to inform contractors and tree planters of the pesticides that had been applied to the seedlings they were planting, and of the associated precautions that should be taken in handling treated stock. A notification stamp was placed on each transport carton which registered the last pesticide applied and the application date, as well as a warning notice to wash hands, avoid storing food or clothing in the cartons, and to wash and change gloves regularly. This is the only notification of safety precautions or guidelines provided to a tree planting contractor. There are currently no guidelines included in a tree planting contract for handling pesticide treated stock. A detailed pesticide application history outlining the pesticides used, their application date, the method and rate of application, and concentration used for the previous 12 months is also attached to the transport cartons, and made available through the Regional/District offices of the Ministry of Forests, Silviculture Branch. A detailed history of pesticide application is also made available to the contractors during a pre-work conference, prior to initiating the start of a

contract. Once the contractor receives this document, it is supposed to be posted and readily available within the tree planting camp (G. Shrimpton, personal communication 1991).

In addition to the above notification of pesticide use and the suggested precautions stamped on the seedling boxes, a pamphlet entitled "Treeplanters' Exposure to Seedling Fungicides by Skin Contact and Inhalation" was prepared by B.C. Research to summarize the findings of their 1988 research and to provide some guidelines to reduce a tree planter's exposure to fungicides.

PESTICIDE EXPOSURE DURING REFORESTATION

It is generally assumed that the amount of pesticide residue remaining on a tree seedling diminishes to an insignificant level by the time of planting. Fungicide residue on tree seedlings in British Columbia nurseries has been found to be within acceptable limits, although highly variable between seed lots (B.C. Research, 1986; 1988). The range of fungicide residue on seedlings measured by B.C. Research (1988) at the time of planting are as follows: 4.2 to 40.0 µg Captan/seedling, 6.3 to 341.3 µg chlorothalonil/seedling, and 3.4 to 106.2 µg benomyl/seedling. Inhalation and dermal exposure of tree planters and nursery employees to particular fungicides has also been found to be minimal and well within acceptable limits, although highly variable among individuals (B.C. Research, 1986; 1988). The range of both captan and chlorothalonil levels measured in the breathing zone was 0.01 to 0.15 $\mu g/m^3$. Dermal exposure on the hand and wrist ranged from 0.02 to 11.4 $\mu g/m^3$ for Captan, 0.04 to 12.5 $\mu g/m^3$ for chlorothalonil, and 1.2 to 22.4 $\mu g/m^3$ for benomyl (B.C. Research, 1988). It would appear from these measurements that the primary source of fungicide exposure for a tree planter would be dermal contact with fungicide residue.

A tree planter's dermal exposure to fungicide occurs primarily on the tree-handling arm and hand, however the shovel-handling arm and hand may contact seedlings while

restocking carrier bags from seedling boxes or while transfering seedlings from storage bags to side bags for planting (B.C. Research, 1988). Inhalation exposure may arise from dislodging foliar pesticide residue during handling of seedlings. This would be most likely during removal of seedlings from transport boxes, when reaching into the box brings the face into close proximity to the seedlings and subsequently the dislodged residue (B.C. Research, 1988).

Apol and Thoburn (1976a-f) performed an series of evaluations of the health hazard posed to nursery workers and tree planters in the Pacific Northwest from the pesticide Thiram (tetramethylthiuram-disulfide). Although air sampling yielded non-toxic levels of Thiram, the authors found a high degree of variation in the concentration of Thiram residue on tree seedlings. Apol and Thoburn (1976a) reported that symptoms of Thiram intoxication could only be attributed to Thiram exposure by general medical history, since there was no statistically significant difference in the incidence of symptoms between groups exposed to Thiram and those not exposed. Attribution of systemic symptoms (headache, dizziness, nausea, diarrhea, stomach complaints, fatigue, and intolerance to alcohol) to Thiram exposure occurred when workers were exposed to trees with a high concentration of Thiram, or when the work week exceeded five days (Apol and Thoburn, 1976a). It was found that Thiram was detectable in the blood of tree planters, even though the breathing zone levels were lower than the prescribed standards (Apol and Thoburn, 1976b,c, and d).

Duell and Morton (1987) presented a more recent case study of a Mexican tree planter suffering from Henoch-Schonlein purpura, which was induced by occupational exposure to Thiram. Tree seedlings had been treated with a 42% solution of Thiram. No indication of the reentry interval was provided.

Banister *et al.* (1990) reported large variability of tree planter susceptibility to pesticide exposure as indicated by PChE inhibition. Some individuals demonstrated depressed PChE activity while others did not after planting the same seedling stock on the same day.

Furthermore, it has been noted that pharmacologically significant absorption may result from seemingly trivial exposure (Morgan, 1980). Given the variability reported for fungicide residue on seedlings and the dermal and breathing zone exposure, the possibility of significant absorption of pesticide can not be eliminated. The likelihood of significant absorption by a tree planter is increased by the difficulty in maintaining adequate personal and occupational hygiene under prevailing primitive camp conditions.

Corrao *et. al.* (1989) studied the hospital admissions of 26,000 licensed pesticide users in Italy. These authors reported a higher risk for tumors of the nervous system and hematopoietic tissues in occupations devoted to forest tree plantation, although the risk was not statistically higher than that for other areas.

HYGIENIC DIFFICULTIES IN THE FIELD

Tree planting in British Columbia is a physically demanding occupation that occurs on a seasonal basis through the warmest part of the year. This may be significant in increasing a tree planter's absorption of pesticide in light of research by Richter *et. al.* (1980), who suggested that heat stress potentiates pesticide absorption due to increased peripheral vasodilation and sweating. Increased organophosphate absorption accentuates the sweating response thereby contributing to further increases in absorption as well as interfering with normal thermoregulation. The resultant depression of the central nervous system and decreased psychomotor function are certainly detrimental to the health and performance of a tree planter.

Also contributing to an increased probability of pesticide absorption, is the prevalence of mucous membrane irritation and dermatitis (Smith, 1987) both of which would increase the absorbed dose for a given exposure level. Contact dermatitis is often refered to as "spruce rash" by tree planters and is common when planting sharp needled seedlings such as spruce. Irritation of mucous membranes may be a reaction to dust, allergen, or chemical exposure.

Prior to 1987, there were no official guidelines for camp standards which would provide for sanitary conditions and promote good hygiene among tree planters. In 1987 the B.C. Ministry of Forests specified standards for tree planting camps to be adhered to by all silviculture contractors. These standards specified careful supervision of: water supply, campsite location, notification of communicable disease, kitchen and meal preparation, dining room cleanliness, food handler hygiene, food quality, food preparation equipment, sanitary facilities, garbage and sewage disposal, and dry rooms (Smith, 1987). The acceptance and provision of these guidelines has done much to increase the maintenance of good personal and occupational hygiene, however, the fact remains that tree planting camps are still primitive by most standards. Tree planters reside in small tents where work clothes are often stored in the same space in which they sleep and store leisure clothing and other personal effects. Shower facilities, although provided, usually lack adequate water pressure and sufficient hot water. Laundry is often done by hand and work clothes are often washed with leisure clothes.

The field conditions during a work day and the piece-work method of payment do not readily provide for distinct rest breaks during which a planter may wash prior to eating a meal. The piece-work system also promotes "cutting corners" to optimize planting efficiency, encouraging tree planters to find the fastest, not the safest, way to plant trees (Davis, 1981).

METHODOLOGY

HYPOTHESES

1) Serum cholinesterase and erythrocyte cholinesterase activity are biochemical indices positively correlated with an estimate of pesticide exposure dose in tree planters during a season of planting work. This estimate of exposure dose is to be based upon separate measures of the reentry interval (time between pesticide application and planting of seedlings), the productivity of the individual tree planter, and seedling species.

2) The cumulative effect of repeated low level pesticide exposure during a planting season may be demonstrated in a significant inhibition of a tree planter's erythrocyte or serum cholinesterase activity serially measured throughout a tree planting season, and/or a depression of motor nerve conduction velocity in a worker's forearm at the end of a planting season compared with conduction velocity after several months recovery from planting work.

3) The change in serum cholinesterase activity, erythrocyte cholinesterase activity, and/or the neurological function tests described, may be positively correlated with an increase in health complaints among exposed tree planters.

SUBJECTS

From a camp of approximately thirty-five tree planters, seventeen volunteers formed an experimental cohort for the serial study of cholinesterase activity. Attrition from the original cohort was 53 percent. Nine subjects remained active participants in the study until the end of the planting season. Two main reasons for attrition were: 1) several subjects ended their planting season prematurely, and 2) some subjects withdrew from the study due to a developing unwillingness to give blood samples. The cohort comprised fourteen

male and three female volunteers between the ages of nineteen and thirty-eight. Previous tree planting experience ranged from nil to fifteen years, with six first year planters and eleven experienced planters.

Thirteen subjects volunteered for a postseason nerve conduction test. Four of these individuals were from the subject pool who had previously given serial blood samples for PChE and AChE analysis, while nine were from various other tree planting camps within B.C.. Due to relocation, extended vacation outside of Canada, or emigration, several of these subjects were unavailable for further testing after several months of recovery from tree planting. Six subjects returned for recovery testing prior to returning to work in the following tree planting season.

FIELD LABORATORY

The tree planting camp of the study group was located approximately 700 kilometers from Vancouver, in the Revelstoke valley region. Camp facilities included a cook trailer, large dining tent, a coed shower tent and pit toilets. Tree planters slept in self-provided shelters, which were typically two man tents. Meals were provided regularly with sufficient variety to satisfy both vegetarian and carnivorous diets. Water was drawn by a gasoline powered pump from a nearby river to provide running water for washing and showering, although lack of water pressure was a recurring problem. A propane heater was used to heat shower water, however the heated water volume was limited and the water filter was subject to frequent clogging. This contributed to showers of short duration that were periodically interrupted by loss of water pressure and cold water temperature. Drinking water was provided in large barrels for camp consumption. A gasoline powered generator provided 120 volt electrical power for the cook trailer, dining tent and laboratory facility. Camp employees included: one contractor, three foremen, approximately thirty-five tree planters, one cook and one maintenance person.

A field laboratory measuring three metres by two metres was constructed in the tree planting camp using a wooden stud frame and plastic tarps. The laboratory was equipped with light and a centrifuge. This laboratory was used for blood drawing, processing of blood, collection of questionnaires, and for storage of equipment during the investigator's absence from the field.

MEASUREMENT OF CHOLINESTERASE ACTIVITY

Blood samples were drawn by venipuncture from the antecubital vein into vacutainer blood tubes containing EDTA(K_3) as an anticoagulant, and stored on ice until analysis. Whole blood cholinesterase activity and serum cholinesterase activity were determined for each sample by the method of Ellman et al. (1961) using acetylthiocholine as substrate and 2,3-dithiobisnitrobenzoate (DTNB) as an indicator reagent. The rate of change in absorbance at 412 nanometers (ΔA412) was determined using a Beckmann DU-8 spectrophotometer with a kinetics module. Erythrocyte cholinesterase activity was determined as the difference between whole blood cholinesterase activity and serum cholinesterase activity as indicated by Voss and Sachsse (1970). Hematocrit and red cell count for each sample were concomitantly determined. Erythrocyte cholinesterase activity was defined in µmoles of substrate hydrolyzed per minute per red cell, while serum cholinesterase activity and whole blood cholinesterase activity was defined in µmoles of substrate hydrolysed per minute per milliliter of serum and per milliliter of whole blood respectively.

The standard curve of ΔA412 against enzyme activity units yielded a highly significant correlation (r^2=0.996).

MEASUREMENT OF NERVE CONDUCTION VELOCITY

A Grass S-9 stimulator and Grass P15 differential preamplifier were used with a Tektronix T912 10MHz storage oscilloscope to determine motor nerve conduction velocity.

Measurements were stored on 1/4 inch tape using a Hewlett Packard 3960 Instrumentation recorder. A schematic representation of the equipment used for conduction velocity determinations is shown in Figure 2. Motor nerve conduction velocity was determined as described by Ma and Levison (1983) for the median and ulnar nerves on both the tree-handling arm and the shovel arm. Surface electrodes were used for stimulation of the respective motor nerves. The median nerve was stimulated first at the wrist between palmaris longus and flexor carpi radialis tendons near the second most distal crease, and secondly at the elbow medial to the brachial artery and the biceps tendon. The ulnar nerve was stimulated similarly first at the wrist medial to the flexor carpi ulnaris tendon near the second most distal crease, and secondly at the elbow just proximal to the ulnar groove. Muscle action potentials were measured for both the ulnar and the median nerves with the active electrode secured over the belly of abductor pollicis brevis, the reference electrode secured over the tendon of abductor pollicis brevis, and the ground electrode on the back of the hand. This electrode placement deviates from the recommendations of Ma and Levison (1983) for the ulnar nerve recording; however, it allowed measurement of muscle action potentials due to both ulnar and median nerve stimulation with a single placement of recording electrodes. Distances were measured on the skin along the shortest path between the two stimulation sites using a flexible metal tape measure.

Due to the impracticality of obtaining serial or repeated measures of nerve conduction velocity in a field study of this nature, neurological function tests were made on tree planters immediately at the season's end, and at least two months after a subject had ceased tree planting, prior to their returning to planting the following season. These tests were performed in a University laboratory.

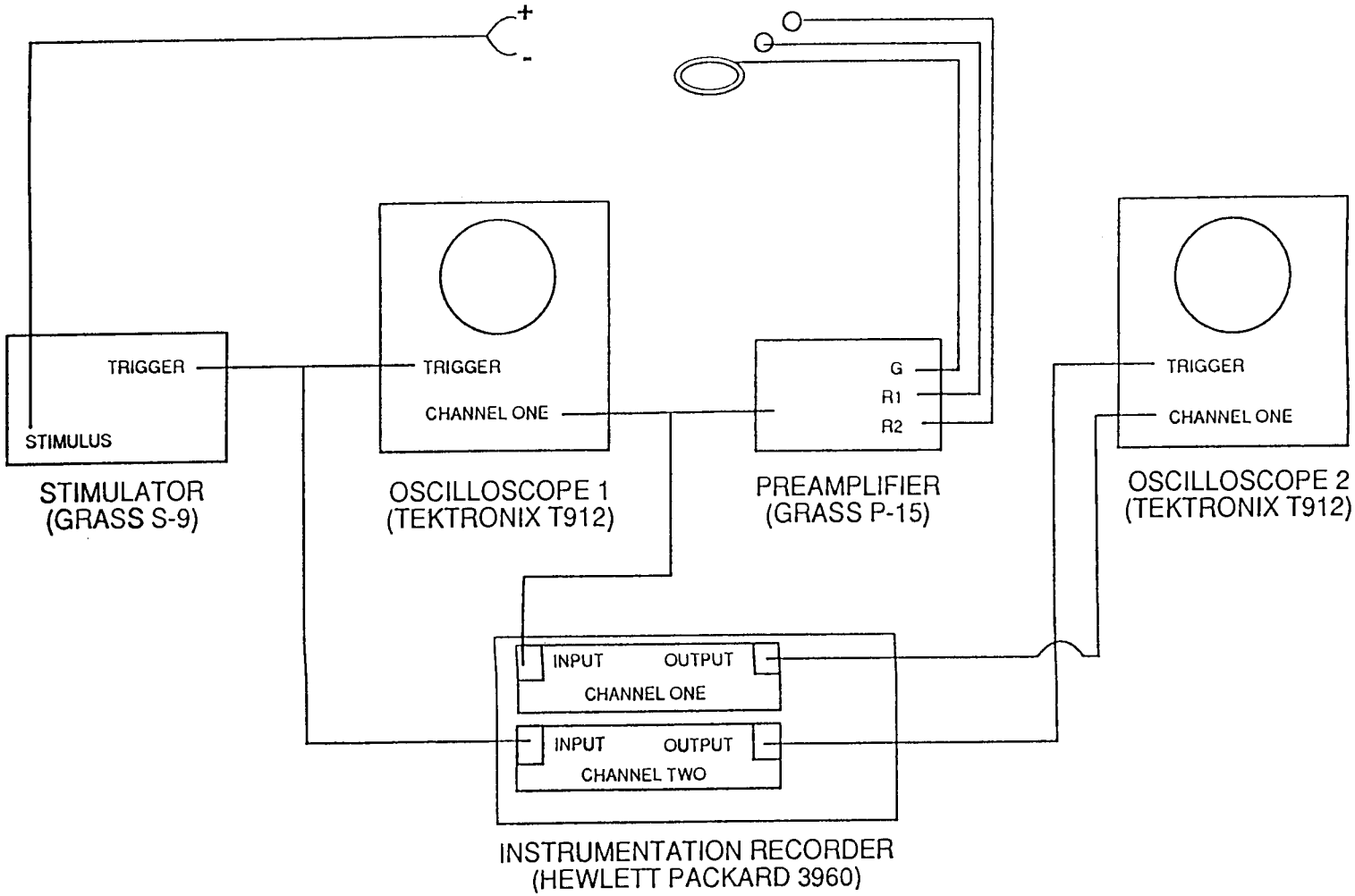

Figure 2 Schematic diagram of apparatus used to measure motor nerve conduction velocity and latency.

EXPOSURE PARAMETERS

Three parameters were quantified which are related to the severity of a tree planter's pesticide exposure. Productivity was defined by the number of trees planted by each subject. Seedling species and the reentry interval were determined for each tree seedling batch planted on a given day.

The number of trees planted from each seedling lot by each subject was recorded in a daily questionnaire kept by the subject, and described below. A pesticide application sheet for each seedling lot was obtained either from the site contractor or directly from the supply nursery in order to identify the specific pesticides used on a seedling batch and the reentry interval for the batch. Several pesticide application sheets were difficult to obtain on-site and were obtained later from a representative of the Ministry of Forests. Reentry interval defines the time between the last anticholinesterase pesticide application and the time of planting.

QUESTIONNAIRES

Three self-report questionnaires were completed by each subject. These consisted of a daily 'work questionnaire' used to assess productivity and hygienic practice of the planter, and a daily 'health questionnaire' to evaluate any health complaints. The number of health complaints by an individual on a given day (HSUM) or during a season of tree planting (total HSUM) was determined. A 'history questionnaire' was completed on the first day of testing to determine the activity that each subject had been engaged in prior to starting the planting season. Copies of these questionnaires are shown in the appendix. The data obtained from them was entered into a Microsoft Excel spreadsheet for processing and analysis.

VIDEO ANALYSIS

Each subject was recorded on video for several minutes during a normal work day, once near the beginning of the research period, and intermittently throughout the season of planting whenever possible. This provided a means to recall at a later date those aspects of tree planting work (personal hygiene, working conditions) that were relevant to pesticide exposure or absorption.

DATA ANALYSIS

Preliminary Statistics

The serial correlation coefficient (P) was calculated for each individual's serially measured AChE, PChE, and WChE activity to determine dependence between successive samples (Kilpatrick, 1973).

The group mean, standard deviation and standard error were calculated for prework and postwork measures of AChE, PChE, WChE, planting productivity, the total HSUM per tree planter per day of testing, and for each of the categorized HSUM values. The group mean difference between prework and postwork activity and the standard error of the difference was calculated for both AChE and PChE. A *post-hoc* paired t-test compared within-day and between-day differences in the above data to determine the significance of any differences observed. A frequency distribution of the number of individuals showing significant AChE and PChE inhibition was determined for each day of testing. Significance was defined by the finding of Callaway *et. al.* (1951) that a 15% and 20% inhibition of AChE and PChE activity, respectively, represents a significant deviation ($p \leq 0.05$) from the normal value.

Cumulative Effects

Time-series analysis was carried out based on the number of days that each individual had been tree planting during the 1989 season. The first day of testing represented the first

day of the series for 11 subjects, the 56th day for four subjects, and the 25th day for one subject.

The cumulative sum (CUSUM) of the difference between mean baseline cholinesterase activity on the first day of sampling (ChE_1) and mean activity on each subsequent sampling day (ChE_i, i = 1 to n) was calculated for both prework and postwork AChE and PChE activity as follows:

$$CUSUM = \sum_{i=1}^{n} [ChE_1 - ChE_i]$$

This analysis provides a means of visualizing time series trends. In general, a rising CUSUM is indicative of an enzyme activity elevation, and a declining CUSUM is indicative of a progressive inhibition of enzyme activity during a tree planting season (Burgess and Roberts, 1980).

Linear and logarithmic regression analyses were applied to the serial AChE and PChE measures to search for significant time-series trends indicative of a cumulative toxic effect resulting from chronic low-level pesticide exposure. Significance was determined using the Pearson product-moment correlation coefficient, r (Cozby, 1981). An analysis of residuals was performed on data indicating significant time-series trends.

Exposure-Effect Association

The association between several exposure parameters and inhibition of WChE, PChE, and RBC AChE was assessed using a chi squared contingency table. The daily change in cholinesterase activity (ΔChE) for each individual was quantified as the percent difference between prework activity and postwork activity defined as:

$$\Delta ChE = ((PRE-POST)/PRE)x100.$$

The percent difference was coded as 'NO EFFECT' when less than 20% for whole blood ChE and plasma ChE, and when less than 15% for RBC AChE. The percent difference

was coded as 'EFFECT' when 20% or greater for whole blood ChE and plasma ChE, and when 15% or greater for RBC AChE. These categories are based on the level of inhibition deemed significant by Callaway *et. al.* (1951).

Exposure parameters were similarly given numerical codes which were determined *post hoc* to represent the full range of observed data. Reentry intervals were determined and coded for four different intervals. The organophosphate reentry interval (OPRE) was defined as the period between the last application of organophosphate pesticide to the seedlings and the day of planting. Similarly, the carbamate reentry interval (CBRE) was defined as the period between the last application of carbamate to the seedlings and the day of planting. The interval between the last application of an organophosphate or carbamate pesticide and cold storage of the treated seedlings was called OPCLD or CBCLD respectively. Coding for these parameters was performed as outlined in Table 1. Seedlings were all coded by species as 'spruce' or 'fir'.

A contingency table was arrayed for each combination of coded WChE, PChE, and AChE against the coded data for productivity, OPRE, CBRE, OPCLD, CBCLD, and seedling species. A chi squared statistic determined the association between inhibition of cholinesterase activity and the above exposure parameters. A linear correlation determined the direction of association between reentry indices and cholinesterase effect.

Table 1 Coding scheme for reentry intervals and productivity.

Parameter	Code	Category Range
OPRE	1	OPRE < 260
(Day)	2	260 ≤ OPRE < 365
	3	365 ≤ OPRE
CBRE	1	CBRE < 240
(Day)	2	240 ≤ CBRE < 365
	3	365 ≤ CBRE
OPCLD	1	OPCLD < 100
(Day)	2	100 ≤ OPCLD < 150
	3	150 ≤ OPCLD
CBCLD	1	CBCLD < 40
(Day)	2	40 ≤ CBCLD < 80
	3	80 ≤ CBCLD <120
	4	120 ≤ CBLD
Productivity	1	200 ≤ Prod < 700
(trees/day)	2	700 ≤ Prod < 1200
	3	1200 ≤ Prod < 1700
	4	1700 ≤ Prod < 2200

Health Complaints

The number of health complaints were summed to yield a 'total HSUM' value for each tree planter on each day of testing. Health complaints were categorized to indicate the relative contribution of musculoskeletal (eg.: back pain, muscle cramping, muscle weakness, joint swelling), vagomimetic (eg.: nausea, diarrhea, brady/tachycardia, abdominal pain), psychological (eg.: depression, irritability, loss of appetite, insomnia), and general complaints (eg.: headache, skin rash, sore throat, coughing). An HSUM value was derived for each categorized complaint in the same manner as for the total HSUM value. The frequency of each complaint and each category of complaint was determined from 66 questionnaires collected from 16 subjects throughout the tree planting season each blood sampling day. Chi square and paired correlational analysis was used to determine the association between the following parameters:

1. Categorized HSUM value per tree planter per season and the prework-postwork percent change in AChE, PChE, and WChE activity.
2. Non-zero categorized HSUM value per tree planter per season and the prework-postwork percent change in AChE, PChE, and WChE activity.
3. Total HSUM value per tree planter per day and the prework-postwork percent change in AChE, PChE, and WChE activity.
4. Categorized HSUM value per tree planter per day and the prework-postwork percent change in AChE, PChE, and WChE activity.

Neural Measures

The group mean and standard error of the mean was determined for each individually determined motor nerve conduction velocity. The difference between the end of season measure and the following pre-season recovery measure was plotted for each individual and each measure to illustrate variability of these data.

RESULTS

Any trend in serially measured AChE and PChE activity resulting from the cumulative effect of chronic low-level exposure to anticholinesterase pesticide was analysed using several methods, which included: a cumulative sum value (CUSUM), linear and logarithmic regression, and the coefficient of serial correlation. Daily prework and postwork measures of both AChE and PChE activity were compared using the student's t-test to identify any statistically significant difference ($p \leq 0.05$) between group mean values throughout the period. The frequency of a significant AChE or PChE inhibition resulting from one day of tree planting, by an individual, was determined. Several other indices of exposure to pesticide, measured from questionnaire response, were each compared separately with the effect on AChE, PChE and WChE activity using contingency table and linear correlation analysis.

CUMULATIVE EFFECTS

The cumulative sum of differences for PChE and AChE between baseline activity on day one and that measured on subsequent sampling days is represented in Figures 3 and 4. These figures illustrate the declining trend in both the prework and postwork PChE CUSUM value, and the rising trend in both the prework and postwork AChE CUSUM value. A negative deviation from zero in these graphs represents a decrease in enzyme activity during the period, and a positive deviation from zero indicates an increase in enzyme activity. Thus, PChE appears to decrease in activity while AChE increases as the planting season progresses. The divergence of the postwork from the prework AChE CUSUM value illustrates the developing effect experienced by humans chronically exposed to low-level pesticide.

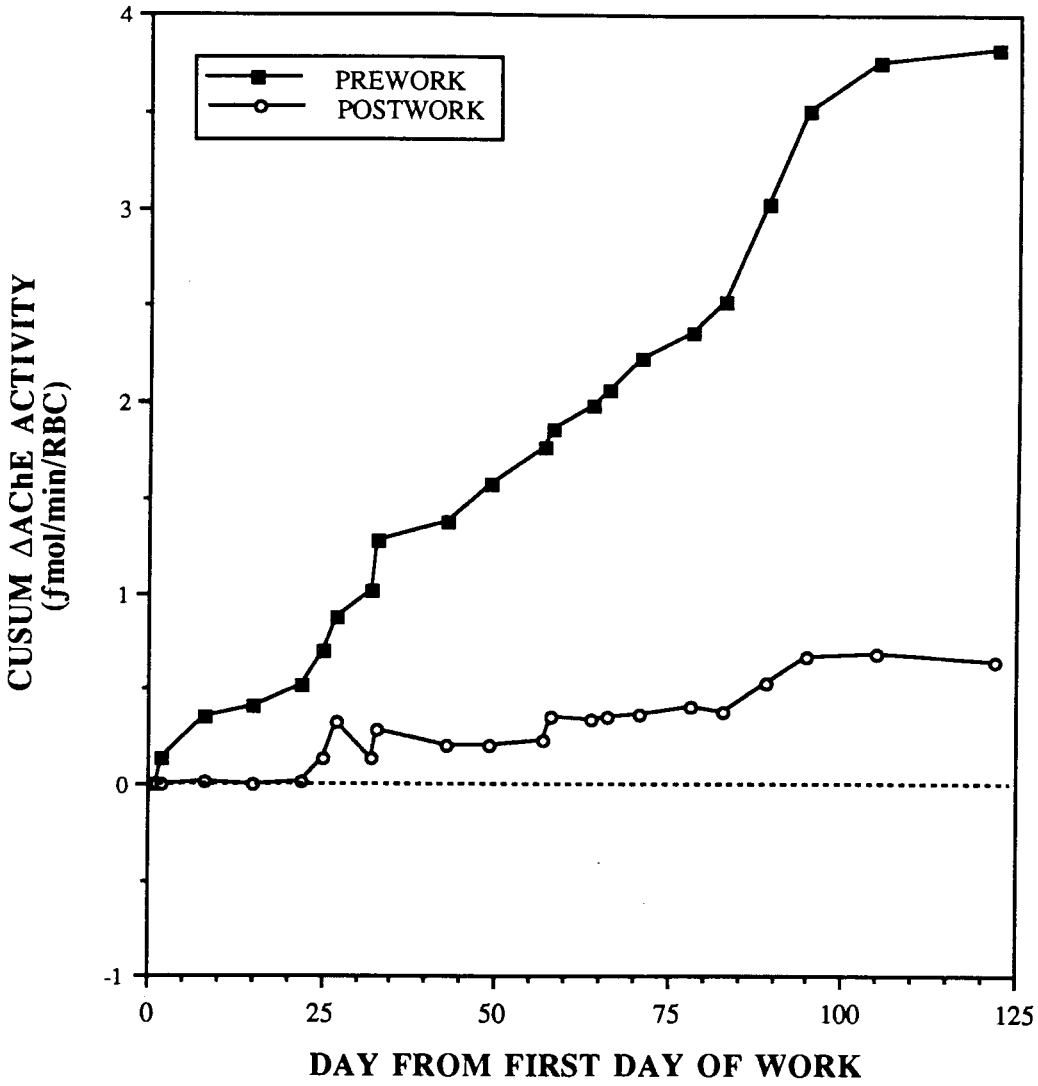

Figure 3 Cumulative sum of the mean prework and postwork difference between baseline and measured AChE activity throughout a tree planting season.

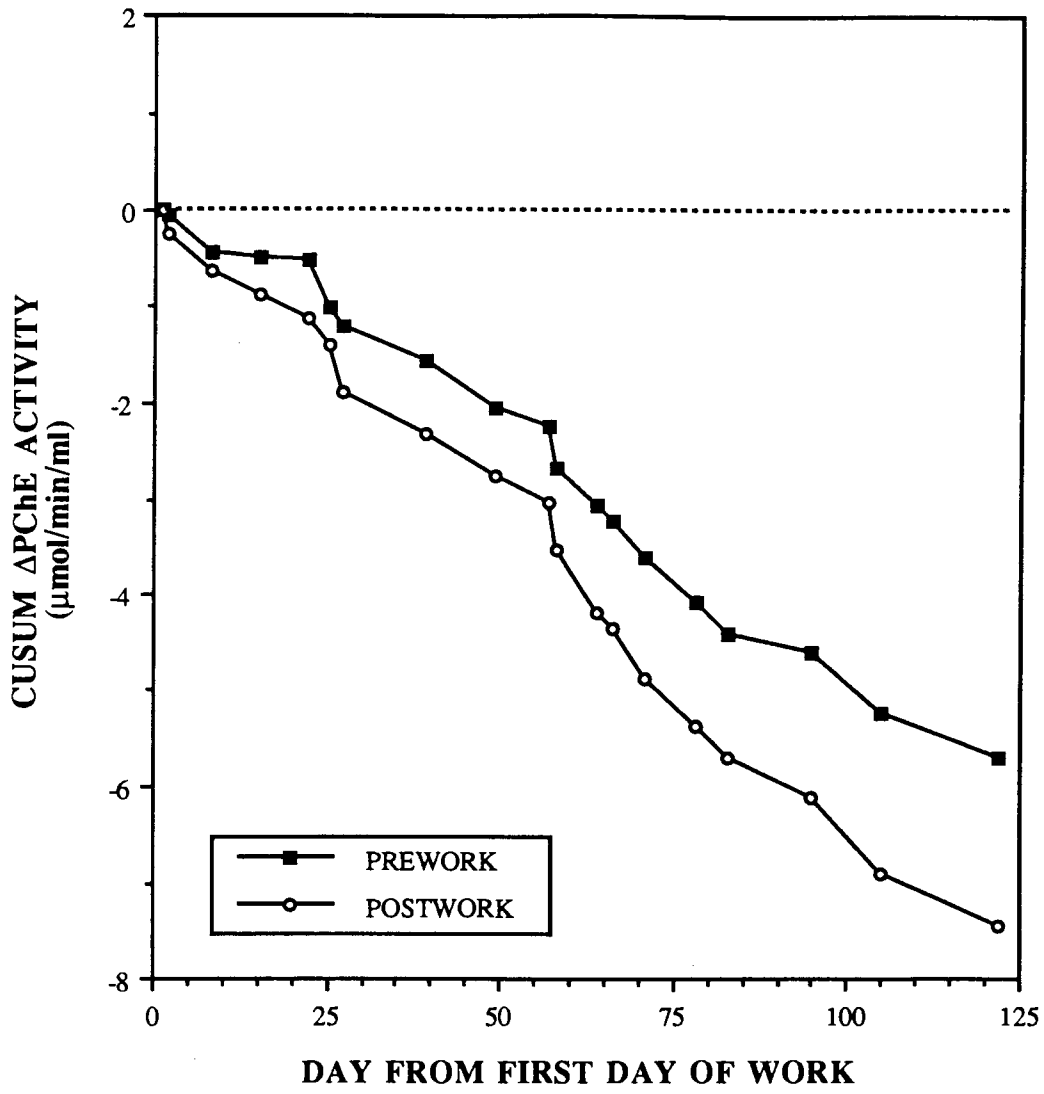

Figure 4 Cumulative sum of the mean prework and postwork difference between baseline and measured PChE activity throughout a tree planting season.

Although postwork AChE activity is only marginally higher as the season progresses, prework AChE activity appears to get progressively higher, relative to the baseline activity on the first day of tree planting. Divergence is also evident between prework and postwork PChE CUSUM values.

A statistically significant trend was found in the linear regression of both prework and postwork serial measures of PChE activity against time, which tended to diminish as the planting season progressed (prework r^2=0.318, n=19, $p \leq 0.01$; postwork r^2=0.392, n=19, $p \leq 0.01$). No statistically significant trend was found in the linear regression of either prework or postwork AChE activity (prework r^2=0.145, n=22, $p > 0.05$; postwork r^2=0.001, n=22, $p > 0.1$).

A logarithmic or exponential regression is physiologically more appropriate than linear regression of AChE and PChE activity versus time, since enzyme inhibition typically demonstrates saturation kinetics for a low-level inhibitor dose (Zubay, 1983). Logarithmic regression analysis (see Figures 5 and 6) yielded a better fit of the PChE serial data versus time than did linear or exponential regression (prework r^2=0.869, n=7, $p \leq 0.01$; postwork r^2=0.724 ,n=7, $p \leq 0.01$). Regression of prework AChE revealed a small increase above baseline activity ([AChE] = 1.052 + 0.0717 Log_{10}[Day] ; r^2=0.659, n=8, $p \leq 0.05$) and postwork AChE data showed no significant trend in time (r^2=0.009, n=9, $p > 0.1$). A plot of PChE residuals (see Figure 7) indicated no underlying time-series pattern in the data, supporting the validity of the fitted curves.

Although visual inspection of CUSUM values and the statistically significant correlation of the logarithmic regression of PChE activity during the tree planting season seem to indicate the presence of a time-series trend in group mean data, the coefficient of serial correlation did not indicate a statistically significant trend in time or any short term cyclic influence in any individual for any of the cholinesterase measures (see Table 2). The null hypothesis of serial independence could not be rejected, which allowed serial data to be considered independently in the analysis of daily exposure effects (Kilpatrick, 1973).

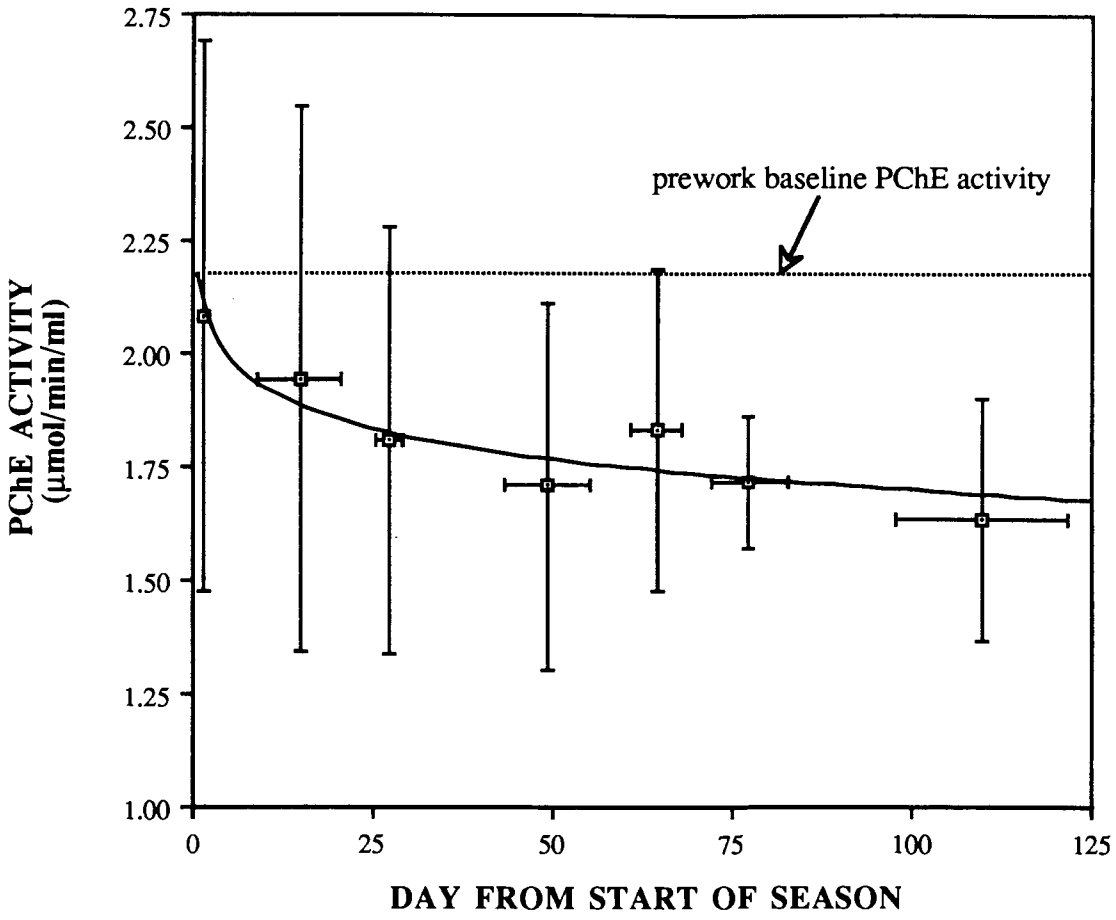

Figure 5 Scatter plot and logarithmic regression curve ($r^2 = 0.869$) of prework PChE activity during a tree planting season in a mixed group (n=16) of male and female tree planters. Error bars represent one standard deviation from the mean.

Equation of the line: $PChE_{(pre)} = 2.1474 - 0.2259 \log_{10} [Day]$.

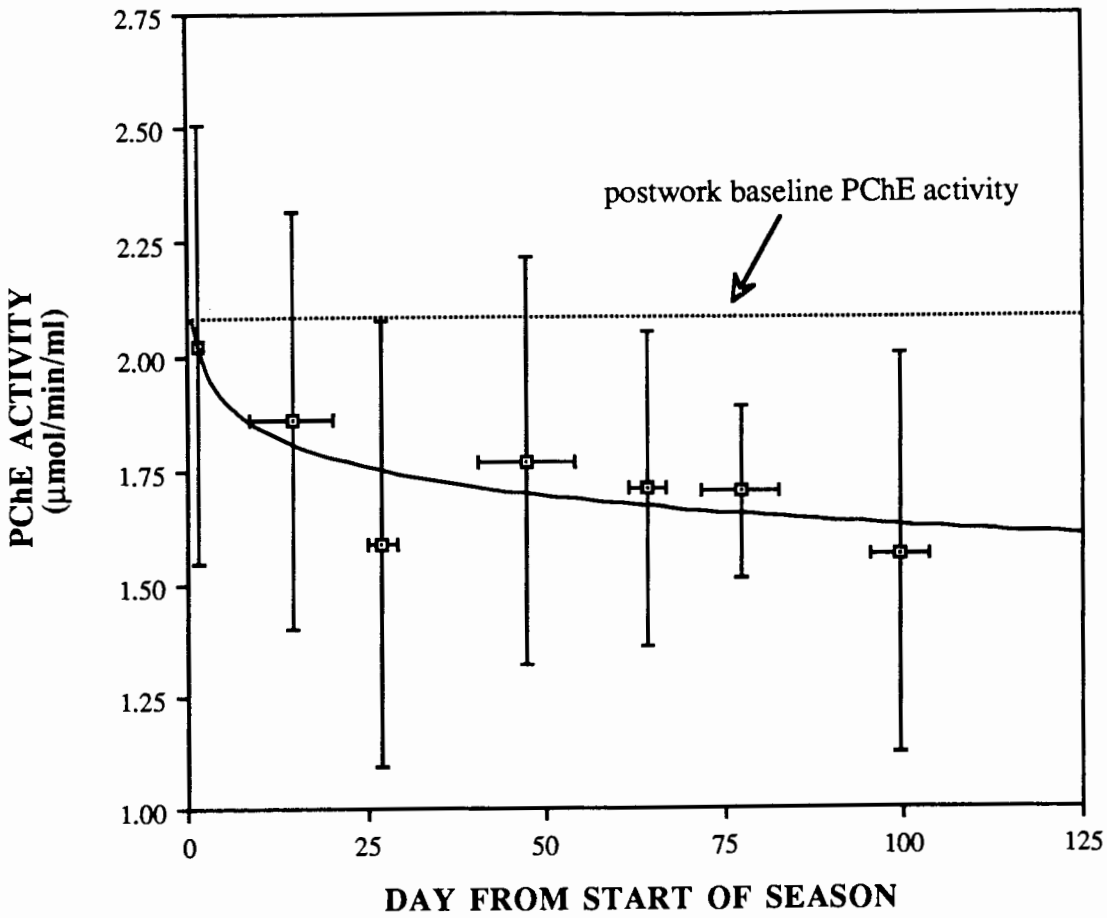

Figure 6 Scatter plot and logarithmic regression curve ($r^2 = 0.724$) of postwork PChE activity during a tree planting season in a mixed group (n=16) of male and female tree planters. Error bars represent one standard deviation from the mean.

Equation of the line: $PChE_{(post)} = 2.0553 - 0.2145 \log_{10} [Day]$.

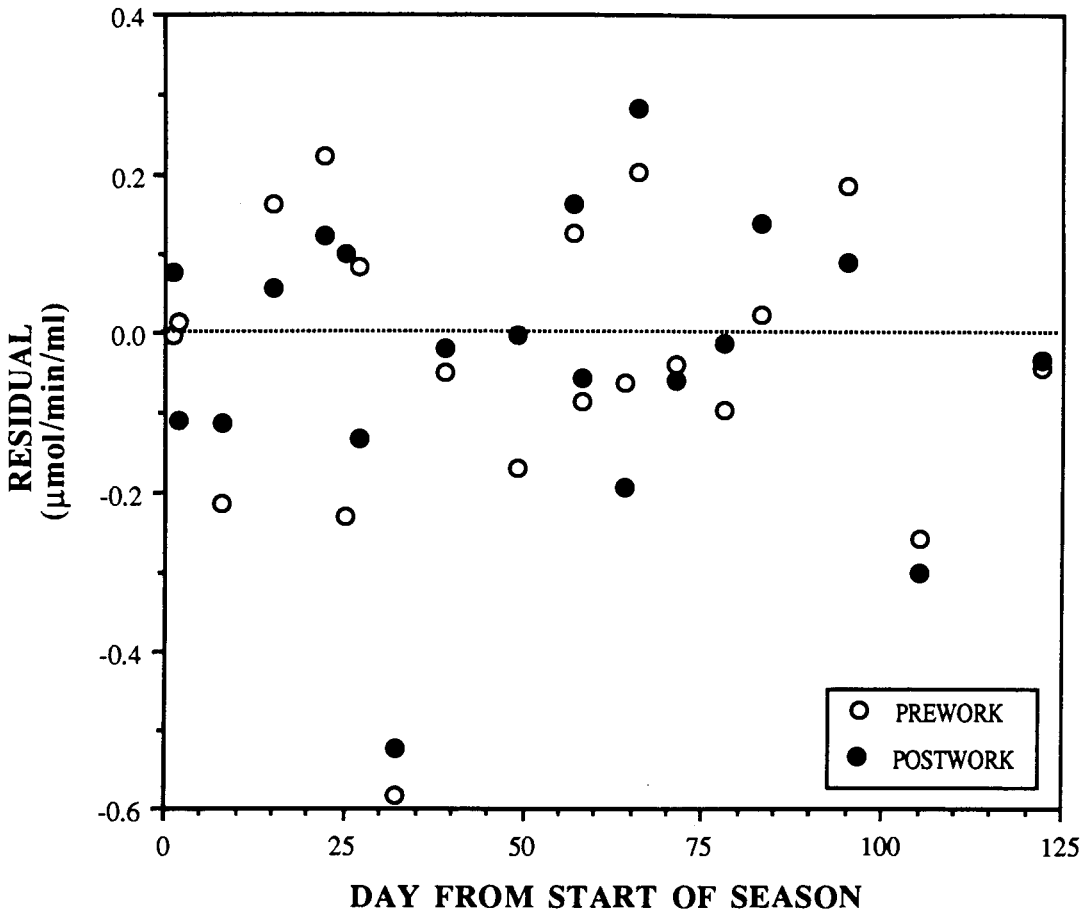

Figure 7 Residuals plot for both the prework and postwork difference between the actual and predicted PChE activity based on the logarithmic regression curves of Figures 4 and 5.

Table 2 The coefficient of serial correlation (P) for prework (Pre) and postwork (Post) AChE, PChE, and WChE activity in sixteen individual tree planters measured several times (n) during a tree planting season.

	AChE				PChE				WChE			
	Pre		Post		Pre		Post		Pre		Post	
Ss	n	P	n	P	n	P	n	P	n	P	n	P
BR	6	1.24	6	2.00	6	1.64	7	1.30	6	1.22	7	1.53
BS	2	-	5	2.47	2	-	5	1.40	2	-	5	2.60
CN	8	1.05	8	1.30	7	2.19	7	2.13	8	2.26	8	2.48
CF	4	3.10	4	1.21	4	3.23*	4	1.37	4	3.04	4	2.53
DW	7	2.97	8	3.16*	7	2.62	8	2.37	7	2.86	9	2.74
DB	5	1.89	7	2.72	5	1.62	7	1.79	5	2.42	7	1.81
DG	7	2.50	9	2.75	6	1.05	8	1.60	7	2.77	8	2.99
DH	5	1.84	4	3.19	5	2.03	4	2.12	5	2.14	4	2.45
GF	3	-	3	-	3	-	3	-	3	-	3	2.45
JW	7	1.74	8	1.39	7	2.80	8	2.11	8	1.99	9	1.84
KM	3	-	2	-	3	-	2	-	3	-	2	2.00
KA	8	1.96	8	2.01	8	1.81	8	2.00	9	2.22	9	1.84
MB	6	2.19	6	1.16	6	2.70	6	2.01	7	2.12	7	1.31
MA	8	2.19	7	2.17	7	1.83	8	1.42	8	1.39	9	2.13
PE	8	1.82	7	2.49	8	2.60	7	0.95	9	1.54	8	2.62
RB	6	0.70*	6	1.67	5	2.25	5	1.89	5	1.79	5	3.09

* P falls outside the range of independence, indicating either a trend in time (RB) or a short term cyclic influence on the measurement (CF, DW) (Kilpatrick, 1973).

CHOLINESTERASE INHIBITION

A significant ($p \leq 0.05$) inhibition of group mean RBC AChE activity was observed on May 17 (day 8) and July 6 (day 58). Group mean plasma PChE was significantly inhibited on May 11 (day 2), May 31 (day 22), and July 23 (day 75). The mean difference between prework and postwork cholinesterase activity, the number of paired prework-postwork samples per day, and the level of significance for each day of testing are listed in Table 3.

Table 3 Mean prework - postwork difference (Mean Δ) for RBC AChE (*f*moles substrate·min^{-1}·RBC^{-1}) and Plasma PChE (μmoles substrate·min^{-1}·ml^{-1}).

	AChE			PChE		
Date	n	Mean Δ	t-prob*	n	Mean Δ	t-prob*
May 10	12	-0.078	.121	11	-0.018	.376
May 11	6	0.028	.306	6	0.113	**.026
May 17	14	0.095	**.004	15	0.038	.253
May 24	13	-0.028	.284	14	0.140	.089
May 31	12	0.021	.375	13	0.119	**.003
June 5	10	-0.040	.136	9	0.196	.065
June 10	4	0.095	.321	1	0.448	-
June 16	2	-0.110	-	2	0.178	-
July 6	9	0.070	**.015	9	-0.011	.453
July 23	5	0.039	.317	6	0.097	**.008

* One-tailed, paired t-test.

** Significant prework-postwork difference (inhibition) at $p \leq 0.05$.

The number of individuals showing significant inhibition of AChE and/or PChE activity for each day of testing as defined by Callaway *et. al.* (1951) is indicated in Table 4. Erythrocyte AChE was significantly inhibited in 15.9% of the tree planters tested (n=82), while PChE was significantly inhibited in only 4.7% of individuals tested (n=86) on one or more days throughout the period of study.

Table 4 Distribution of individuals showing inhibition of RBC AChE and Plasma PChE.

	% Inhibition of RBC AChE			% Inhibition of Plasma PChE		
Date (Day #)	X≤0	0<X≤15	X>15*	X≤0	0<X≤20	X>20*
May 10	8	1	2	5	6	0
May 11	2	3	1	1	5	0
May 17	4	6	4	5	10	0
May 24	7	4	2	1	12	1
May 31	6	5	0	3	10	0
June 5	6	2	1	1	6	1
June 10	2	0	1	0	1	0
June 16	0	1	0	0	2	0
July 6	1	7	1	6	2	1
July 23	2	2	1	0	6	0
Total	38	31	13	22	60	4

* Significant inhibition at the p≤0.05 level (Callaway *et. al.*, 1951).

EXPOSURE AND CHOLINESTERASE INHIBITION

Worker productivity and several different reentry intervals (OPRE, CBRE, OPCLD, and CBCLD) were investigated as pesticide exposure indices which might reflect a significant inhibition of AChE, PChE, or WChE activity occurring after a day of work. The mean, standard deviation, minimum and maximum values, and number of measures for each index is listed in Table 5. In addition, the seedling species was investigated as a potential exposure index, since species with sharp, firm needles, such as spruce, are more likely to induce skin irritation than soft needled species, such as fir. Of the seedlots studied, there were six spruce and three fir, all of which had been treated with an anticholinesterase pesticide.

Table 5 Descriptive statistics for productivity and reentry intervals.

Parameter	n	Mean	Std. Dev.	Minimum	Maximum
Productivity (tree/day)	70	884	496	200	2100
OPRE (day)	10	331.9	143.2	222	717
CBRE (day)	14	236.6	68.5	182	391
OPCLD (day)	8	178.0	144.7	82	533
CBCLD (day)	10	92.1	67.0	14	211

Note: OPRE = the number of days between the last application of organophosphate pesticide and planting of seedlings.

CBRE = the number of days between the last application of carbamate pesticide and planting of seedlings.

OPCLD = the number of days between the last application of organophosphate pesticide and cold storage of seedlings.

CBCLD = the number of days between the last application of carbamate pesticide and cold storage of seedlings.

Contingency table analysis of coded AChE, PChE, and WChE against coded productivity yielded chi squared values of 10.92 (df=4, $p \leq 0.05$), -0.732 (df=4, $p > 0.05$), and 21.704 (df=4, $p \leq 0.01$) respectively. These data indicate a significant association between productivity and inhibition of AChE and WChE activity.

The correlation between productivity and inhibition of AChE, PChE, or WChE was found to be significant on several days of testing, however, the direction of correlation was inconsistent (see Table 6). On May 10, ΔWChE and productivity were positively correlated ($r^2=0.984$, n=3), while on May 11, ΔAChE and productivity were negatively correlated ($r^2=0.927$, n=4). Change in PChE was positively correlated to productivity on May 17 ($r^2=0.408$, n=8), and negatively correlated to productivity on May 31 ($r^2=0.561$, n=11) and June 5 ($r^2=0.446$, n=8).

Table 6 The correlation coefficient and level of significance for the relationship between worker productivity and inhibition of AChE, PChE, and WChE

	AChE		PChE		WChE	
Date	n	r	n	r	n	r
May 10	4	0.552	5	0.022	3	*0.994
May 11	4	**-0.963	4	-0.874	5	-0.510
May 17	7	-0.080	8	*0.639	7	0.329
May 24	6	-0.453	7	0.033	5	-0.488
May 31	10	0.493	11	**-0.749	8	0.444
June 5	7	0.496	8	*-0.668	6	0.517
June 10	-	-	-	-	3	0.666
Overall	41	-0.039	42	-0.017	43	0.092

* $p \leq 0.10$

** $p \leq 0.05$

Contingency table analysis of coded AChE, PChE, and WChE against coded reentry interval found an association between carbamate reentry interval (CBRE) and effect on AChE (X^2=7.585, df=2, p≤0.05), as well as an association between organophosphate cold storage interval (OPCLD) and PChE inhibition (X^2=5.178, df=2, p≤0.1). No other association between reentry and cholinesterase inhibition was deemed significant (see Table 7). Correlational analysis yielded no significant relationship between any reentry interval and cholinesterase effect (see Table 8).

Chi squared analysis showed no significant association between seedling species and effect on AChE (X^2=1.592, df=1, p=0.21), PChE (X^2=0.881, df=1, p=0.35), or WChE (X^2=1.143, df=1, p=0.29).

Table 7 Chi squared association between reentry indices and effect on cholinesterase activity (% difference between pre and postwork).

	OPRE (df=2)		OPCLD (df=2)		CBRE (df=2)		CBCLD (df=3)	
	X^2	p	X^2	p	X^2	p	X^2	p
AChE	2.41	.30	1.32	.52	7.59	**.02	2.26	.52
PChE	1.76	.41	5.18	*.08	0.83	.66	2.62	.45
WChE	0.48	.79	1.62	.45	2.82	.24	0.38	.94

* p≤0.10

** p≤0.05

Table 8 Correlation (r) of the reentry indices with cholinesterase inhibition (% difference between pre and postwork) in a number of tree planters (n).

	OPRE		OPCLD		CBRE		CBCLD	
	n	r	n	r	n	r	n	r
AChE	51	.141	51	.084	53	.226	53	.105
PChE	40	.055	40	.032	45	.105	45	.084
WChE	48	.161	48	.176	50	.105	50	.141

$p>0.10$ for all cases.

HEALTH COMPLAINTS

The mean number of health complaints (HSUM) per tree planter per day was 1.9 (S.D.= 1.9). The group mean daily HSUM did not differ significantly from day to day. The health complaints comprised 39% musculoskeletal, 14% vagomimetic, 29% psychological, and 18% general complaints (see Table 9). The three most prevalent complaints were joint swelling (16.6%), fatigue (15.2%), and back pain (10.5%) (see Table 10).

Table 9 The mean, standard error of the mean (S.E.M.), and range of health complaints per tree planter per day under each symptom classification. (Data from 66 health questionnaires).

Class of Symptom	Mean	S.E.M.	Min.	Max.
Musculoskeletal	0.8	0.1	0	3
Psychological	0.6	0.1	0	3
General	0.4	0.1	0	4
Vagomimetic	0.3	0.06	0	2

Table 10 Number and classification of health complaints and percent of total complaints reported by a group of tree planters (n=16) in a daily health questionnaire during one tree planting season.

SYMPTOM	COMPLAINTS	PERCENT	CLASS*
JOINT SWELLING	172	16.7	M
FATIGUE	157	15.2	P
BACK PAIN	108	10.5	M
MUSCLE WEAKNESS	76	7.4	M
NOSE RUNNING	54	5.2	V
COUGHING	53	5.1	G
IRRITABILITY	52	5.0	P
NOSE IRRITATION	46	4.5	G
DEPRESSION	44	4.3	P
MUSCLE CRAMP	35	3.4	M
EYE IRRITATION	30	2.9	G
ABDOMINAL PAIN	27	2.6	V
LOSS OF APPETITE	26	2.5	P
NIGHT SWEATS	26	2.5	V
NAUSEA	22	2.1	V
SKIN RASH	21	2.0	G
INSOMNIA	16	1.5	P
HEADACHE	15	1.5	G
SORE THROAT	15	1.5	G
CONSTIPATION	10	1.0	V
DIZZINESS	9	0.9	G
MUSCLE TWITCH	7	0.7	M
BLURRED VISION	4	0.4	V
DIARRHEA	3	0.3	V
Δ HEART RHYTHM	3	0.3	V
NOSE BLEED	2	0.2	G

*M = MUSCULOSKELETAL P = PSYCHOLOGICAL
G = GENERAL V = VAGOMIMETIC

The HSUM data for each testing day during the season were combined and compared to the AChE, PChE, or WChE prework-postwork effect using a contingency table chi squared analysis and correlation. Chi squared analysis indicated a significant association between the number of 'general complaints' and the prework-postwork difference in PChE (X^2=5.88, df=2, p=0.053). This analysis indicated no other statistically significant association between the number of musculoskeletal, psychological, or vagomimetic health complaints and effect on AChE, PChE, or WChE activity. However, there was a significant correlation (r^2=.087, n=62, p≤0.05) between the general complaint HSUM and the PChE effect, and a lesser correlation (r^2=0.065, n=60, p≤0.10) between the musculoskeletal HSUM and AChE effect (see Table 11).

Table 11 Correlation of the categorized HSUM value with the prework-postwork difference in AChE, PChE, and WChE respectively in tree planters throughout a season of working.

Category	AChE r (n=60)	PChE r (n=62)	WChE r (n=64)
Musculoskeletal	*.255	-.103	.184
Psychological	.101	.042	.162
General	-.016	**.295	.124
Vagomimetic	.063	.093	.011

* p≤0.10 level
** p≤0.05 level
*** p≤0.01 level

Since a large number of HSUM values were zero, a correlation coefficient was generated for the non-zero categorized health complaints against the percent change in AChE, PChE, and WChE activity (see Table 12). This analysis was expected to provide additional information regarding the degree of cholinesterase effect when there were health complaints. The only statistically significant correlation found was between the non-zero general HSUM and inhibition of WChE activity (r^2=0.178, n=18, p≤0.10), however, this was not significant at the p≤0.05 level.

Table 12 Correlation of the categorized non-zero HSUM value with the prework-postwork difference in AChE, PChE, and WChE respectively in tree planters throughout a season of working.

	AChE		PChE		WChE	
Category	n	r	n	r	n	r
Musculoskeletal	35	.046	35	-.104	36	-.029
Psychological	24	-.257	25	.233	27	-.127
General	17	-.018	20	.308	18	*.422
Vagomimetic	16	-.000	17	.017	16	-.076

* p≤0.10 level
** p≤0.05 level
*** p≤0.01 level

Both the total HSUM and categorized HSUM data were also analysed for correlation with cholinesterase effect on each individual day of testing. A statistically significant correlation was apparent between total HSUM and the prework-postwork difference in AChE activity on May 11 (r^2=0.760, n=5, p≤0.05) and July 6 (r^2=0.573, n=7, p≤0.05).

The prework-postwork difference in PChE was significantly correlated with total HSUM on May 11 (r^2=0.899, n=5, p≤0.01), June 5 (r^2=0.416, n=9, p≤0.05), and July 23 (r^2=0.691, n=3, p≤0.10). A significant correlation was also found between percent change in WChE and total HSUM on May 11 (r^2=0.762, n=7, p≤0.01). It should be noted that direction of the correlation was not consistent between days, even when comparing only the statistically significant correlations. No other statistically significant correlation was found between total HSUM and the daily prework-postwork difference for AChE, PChE, or WChE activity (see Table 13).

Table 13 Correlation (r) between the total HSUM and the percent change in AChE, PChE, and WChE activity on specific days throughout a season in tree planters.

	AChE		PChE		WChE	
Date	n	r	n	r	n	r
May 10	10	.317	10	.312	7	.325
May 11	5	**-.872	5	***-.948	6	***-.873
May 17	11	.194	12	-.264	11	-.231
May 24	11	-.015	12	.106	10	.058
May 31	11	.294	12	-.336	9	.202
June 5	8	.128	9	**.645	7	.018
June 10	3	-.687	0	-	4	-.130
June 16	1	-	2	-	2	-
July 6	7	**.757	0	-	5	.233
July 23	2	-	3	*-.831	3	.634

* p≤0.10 level
** p≤0.05 level
*** p≤0.01 level

The categorized HSUM for each day was found to be significantly correlated with the percent change in cholinesterase activity on several days of testing (see Tables 14, 15, and 16). There were several days which showed significant correlation between multiple measures. On May 10 there was a significant correlation between the musculoskeletal HSUM and both the AChE and WChE effect, and also between the general HSUM and the PChE effect. On May 11 there was a significant correlation between each of the following: the musculoskeletal HSUM and the PChE effect, the psychological HSUM and the WChE effect, and both the general HSUM and the vagomimetic HSUM with each of the AChE, PChE, and WChE effects. May 31 showed significant correlation between musculoskeletal HSUM and WChE effect, psychological HSUM and AChE effect, and general HSUM with WChE effect. June 10 showed a highly significant correlation between both the psychological HSUM and vagomimetic HSUM and the WChE effect.

Table 14 Correlation (r) between the categorized daily HSUM and the percent change in AChE activity on specific days throughout a season in tree planters.

Date	n	Musculo.	Psych.	General	Vagal
May 10	10	**.638	.191	-.118	.121
May 11	5	-.580	-.615	***-.910	**-.855
May 17	10	.073	-.152	.275	.110
May 24	9	-.311	-.378	.044	-.089
May 31	9	-.392	**.657	-.028	-.316
June 5	7	-.575	.443	.515	-.200
June 10	3	-.766	-	-.342	-

* $p \leq 0.10$ level
** $p \leq 0.05$ level
*** $p \leq 0.01$ level

Table 15 Correlation (r) between the categorized daily HSUM and the percent change in PChE activity on specific days throughout a season in tree planters.

Date	n	Musculo.	Psych.	General	Vagal
May 10	10	.026	.007	*.523	.372
May 11	5	**-.862	-.514	*-.728	***-.967
May 17	11	-.466	-.379	.001	.345
May 24	10	.227	.228	.142	.031
May 31	10	.357	-.435	-.466	.158
June 5	7	.042	.310	.440	.696

* p≤0.10 level
** p≤0.05 level
*** p≤0.01 level

Table 16 Correlation (r) the categorized daily HSUM and the percent change in WChE activity on specific days throughout a season in tree planters.

Date	n	Musculo.	Psych.	General	Vagal
May 10	7	**.779	.256	-.114	-.043
May 11	6	-.559	***-.876	*-.632	***-.876
May 17	10	-.406	-.397	-.107	-.158
May 24	8	-.057	-.372	-.176	-.039
May 31	6	*.674	-.248	**.792	-
June 5	5	-.151	.534	.338	.396
June 10	4	-.567	***.998	.304	***-.998

* p≤0.10 level
** p≤0.05 level
*** p≤0.01 level

A stronger relationship between the number of reported symptoms and the degree of cholinesterase inhibition might be obtained by considering the number of health complaints reported by individuals who demonstrated a clinically significant inhibition of cholinesterase activity (greater than 15% inhibition). A correlation coefficient was calculated for the association between AChE inhibition and the HSUM value for the individuals who showed both a significant AChE inhibition and provided a completed health questionnaire. Since few individuals showed both inhibition of PChE and provided a completed health questionnaire, these data were not analysed. Of the thirteen individuals demonstrating greater than 15% inhibition of AChE, eleven provided a completed health questionnaire for the day in question. No significant correlation was found between any of the HSUM values and AChE inhibition in these individuals. However, a general trend toward an increase in both the total HSUM and the musculoskeletal HSUM seems to follow an increase in the percent inhibition of AChE if the data are viewed using a scattergram and the two data points representing the greatest inhibition are discounted (see Figures 8 and 9).

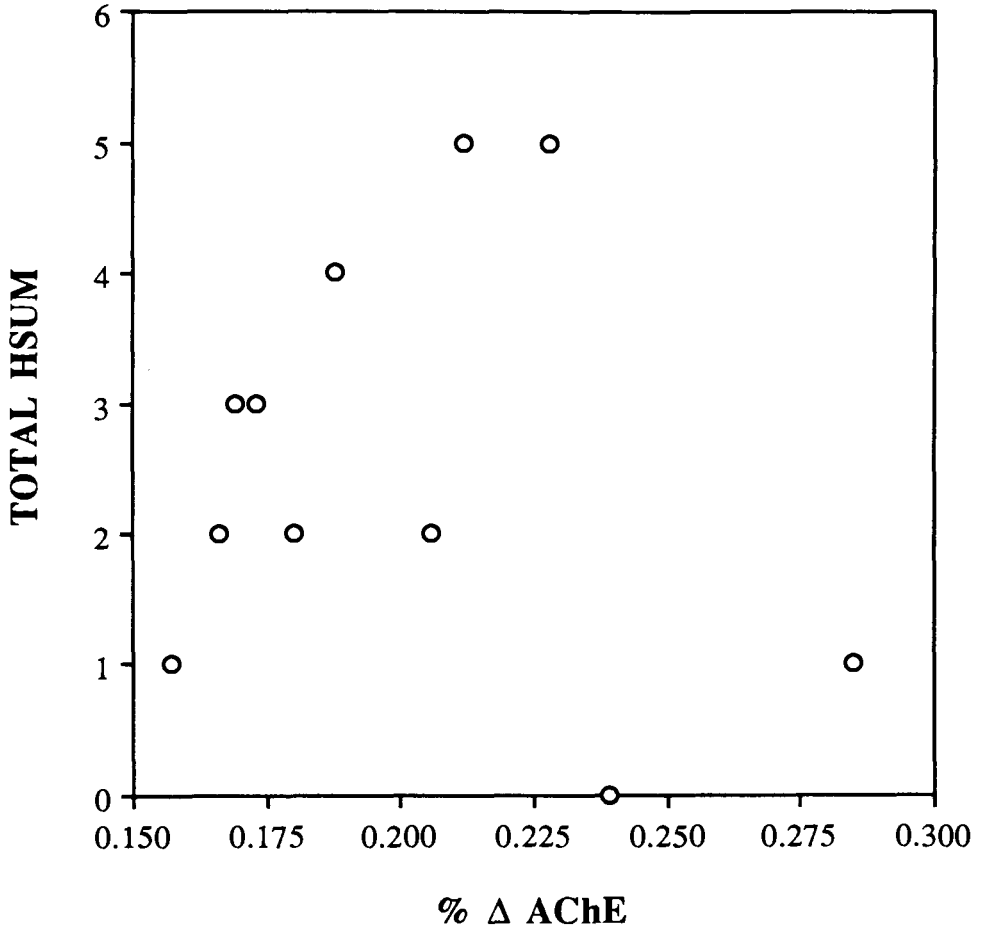

Figure 8 Scattergram of total HSUM and percent change in AChE activity for eleven individuals showing a clinically significant inhibition of AChE activity

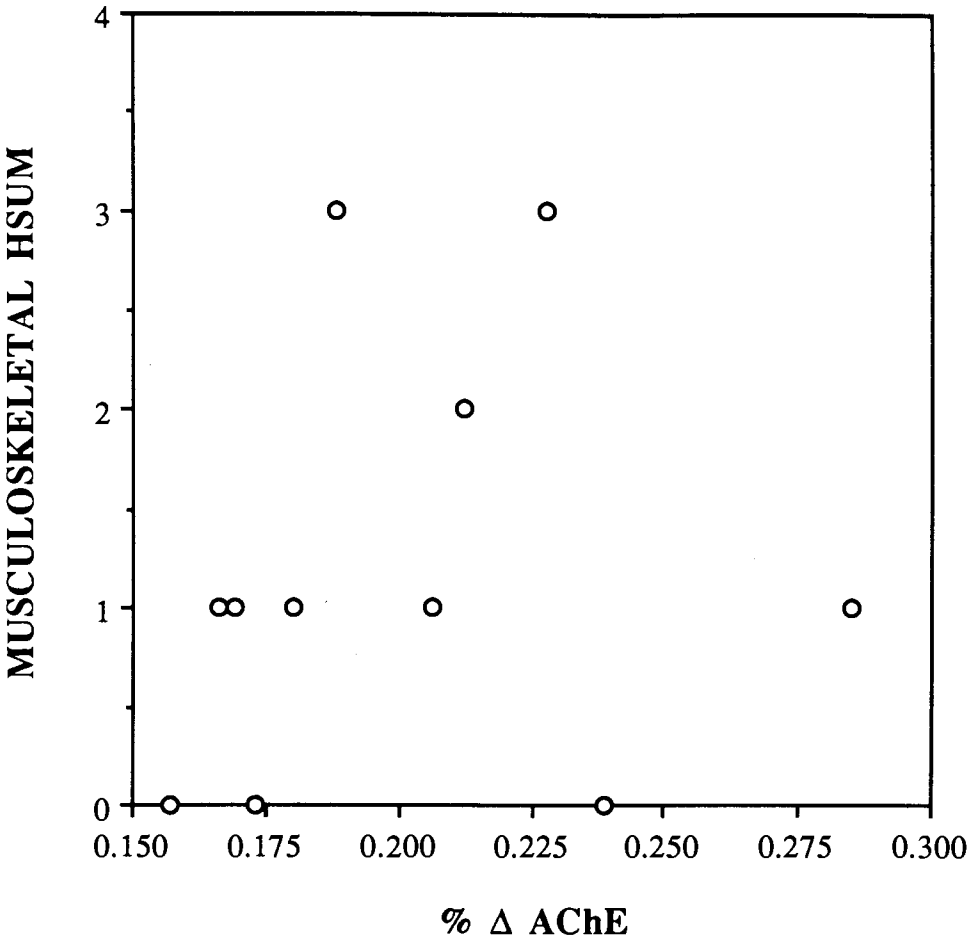

Figure 9 Scattergram of musculoskeletal HSUM and percent change in AChE activity for eleven individuals showing a clinically significant inhibition of AChE activity

NEURAL MEASURES

All measured motor nerve conduction velocities were within the normal range of a healthy adult. Distal latency measurements have been expressed as a conduction velocity to normalize for between test differences in conduction distance. There was no significant change in group mean conduction velocity or distal latency of either the ulnar or median nerves after a post season recovery period of several months (see Figure 10). The recovery interval ranged from 2 months to 8 months with a mean of 5.6 months. There were, however, significant individual changes in conduction velocity between the end of the tree planting season and after several months of recovery in some tree planters. Several tree planters showed an increase in motor nerve conduction velocity while others showed a decrease in velocity after a recovery period of several months (see Figure 11). Some of the inter-test variability may be due to changes in skin temperature between testing sessions, which was not controlled for. Conduction velocity has been shown to vary by approximately 2.5 m/s for every degree change in temperature (Ma and Liveson, 1983).

Repetitive activity was observed after a single stimulus on both end of season and recovery tracings in several individuals. The latency of the repetitive firing was characteristic of the individual, ranging from 100 msec to 350 msec, and showed an amplitude of ten percent or less of the initial evoked M-wave. This latency is considerably longer than the 15 to 30 msec latency reported in other research of repetitive firing (Jager *et. al.*, 1970) which suggests that it is not the same phenomenon described by these authors. Similar long-latency repetitive activity was also observed in tracings from two normal fourth year university students who were not tree planters, suggesting that the late spike is either an artifact of the testing protocol, or is due to a voluntary response to surface stimulation.

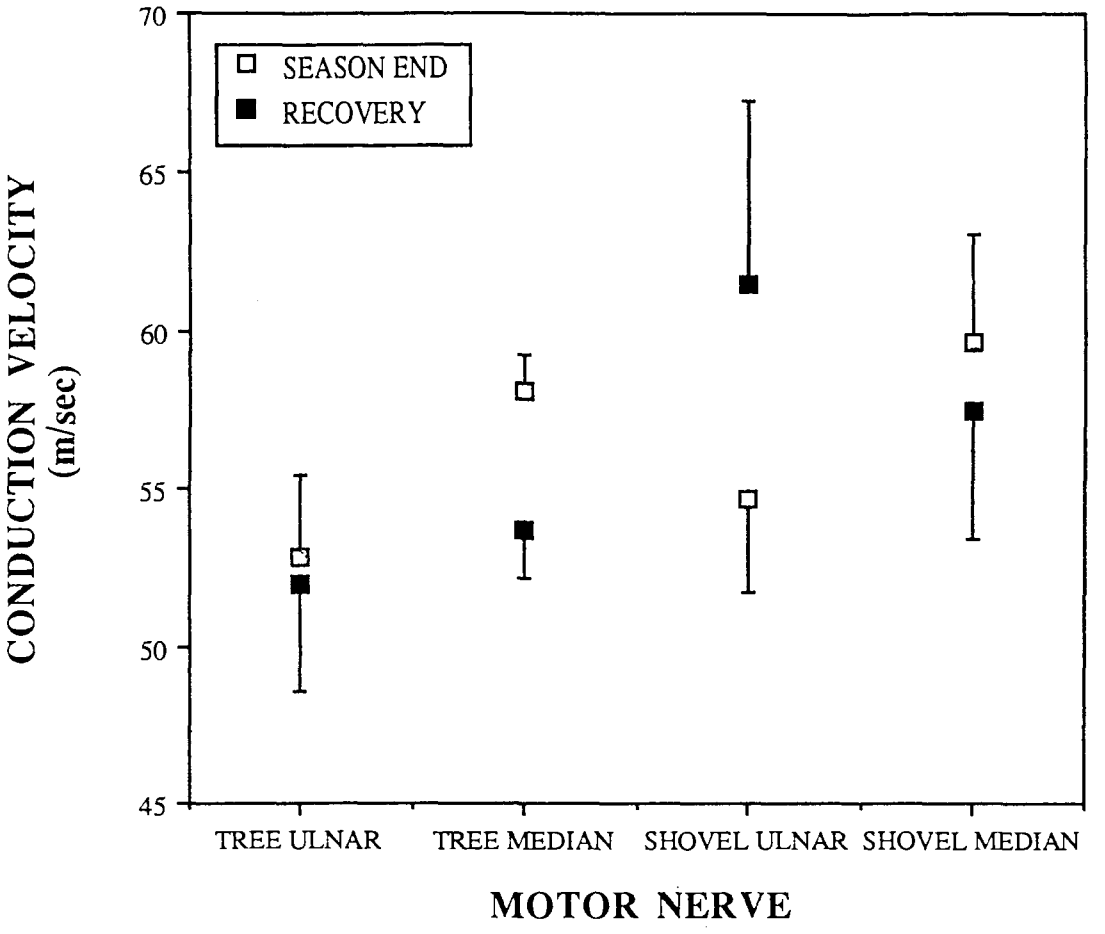

Figure 10 Mean conduction velocity (n=6) for the elbow-wrist segment of the ulnar and median motor nerves measured immediately at the end of a tree planting season and after several months of recovery . Error bars represent standard error of the mean.

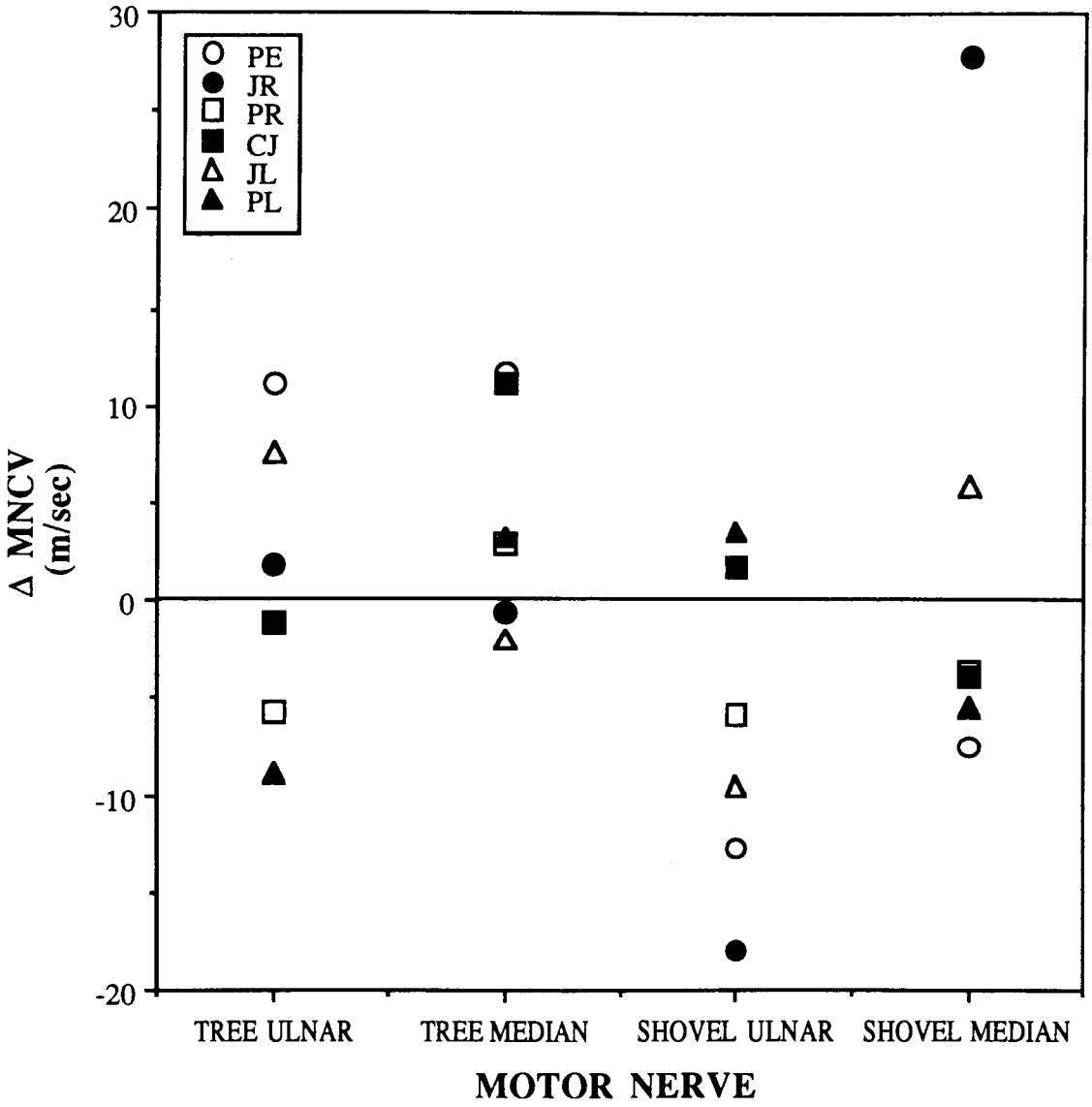

Figure 11 The difference between measurement of motor nerve conduction velocity at the end of a season and after several months of recovery from tree planting work for individual tree planters (ΔMNCV = end season - recovery). Positive values indicate slowing of conduction velocity and negative values indicate increased conduction velocity.

DISCUSSION

GENERAL

The existence of a toxicological hazard to tree planters from organophosphate or carbamate pesticide exposure was confirmed through the serial determination of erythrocyte AChE and plasma PChE activity during a tree planting season. A significant inhibition of group mean erythrocyte AChE activity or serum PChE activity resulting from a day of tree planting work was observed on five of ten serially selected days of testing throughout a planting season in tree planters, although there were no days on which both AChE and PChE were significantly inhibited (see Table 3). This may be due to a differing cholinesterase affinity of the pesticides that were responsible for inhibition on a given day.

It should be noted that the t-test assumption of sample size greater than ten was violated for the analysis of data on several days. Two days on which the sample size was adequate, however, showed significant inhibition ($p \leq 0.01$) of AChE (May 17) or PChE (May 31) (see Table 3).

Plasma PChE is typically regarded as a more sensitive measure of anticholinesterase activity than AChE; however, the frequency of significant inhibition of an individual's AChE activity was considerably higher than for PChE (see Table 4). The proportion of individuals showing some inhibition, although below a level of clinical significance, is considerably greater for PChE (60 of 86 individuals) than for AChE (31 of 82 individuals). Thus, plasma PChE activity, in fact, was more sensitive than AChE to the low level pesticide exposure of workers in this study, which did not result in a level of clinical significance ($\Delta PChE \geq 20\%$, $\Delta AChE \geq 15\%$). If the simple percent inhibition of PChE and AChE activity is considered, 76% of individuals tested showed some degree of PChE inhibition, while only 54% showed AChE inhibition. This could result from the fact that PChE activity is a more sensitive indicator of anticholinesterase activity, or is more

susceptible to a false positive result. The greater frequency of a clinically significant level of AChE inhibition (≥15%) must also consider the potential effect of physical activity on cholinesterase activity. The physical demands of tree planting might be expected to produce an increase in PChE activity and to have little or no effect on AChE activity (Pawlowska *et. al.*, 1985b). Thus a confounding effect of exercise may partially mask inhibition of PChE, resulting in fewer clinically significant cases of PChE inhibition.

PESTICIDE EXPOSURE PARAMETERS

Several parameters that may be associated with an increased exposure or absorption of pesticide have been compared with observed cholinesterase inhibition. A significant association was found between productivity and both AChE and WChE inhibition, however the correlation was significantly positive on some, and significantly negative on other days. Thus it is unwise to speculate on the degree of cholinesterase inhibition, and secondarily on the amount of absorbed pesticide, based solely on worker productivity. Clearly, other factors play a role. It is evident from these data, however, that high productivity is associated with an increased pesticide absorption on some days, and with a decreased absorption on other days. That high productivity should increase exposure to chemicals on the tree seedlings is logical since handling a large number of trees would bring the worker into contact with a larger dose of pesticide. That low productivity should be associated with an increased exposure to pesticide is more difficult to explain. Several secondary factors may play a role in this case. One factor might be the worker's level of experience, which generally is lower for the less productive worker. Experience may influence hygiene, the manner in which tree seedlings are handled, and possibly the total time of direct handling contact with tree seedlings. If a more experienced tree planter uses a more efficient method of handling trees, less total time may be spent in direct contact with the seedling than for an inexperienced tree planter. A further confounding factor in this analysis is the effect exercise has on both AChE and PChE activity. Pawlowska *et. al.*

(1985a, 1985b) found that exercise increased the activity of cholinesterase in the blood. The physical activity of tree planting work may produce a false negative result by acting to modify the effect of pesticide on cholinesterase inhibition.

There was a weak association between carbamate reentry interval (CBRE) and AChE effect, and between organophosphate cold storage interval (OPCLD) and PChE effect, however no significant correlation was found. Although an association between either of the above reentry intervals and AChE or PChE inhibition could not be unequivocally defined, the fact that some association was found indicates that the time between pesticide application and cold storage of seedlings needs to be considered in addition to the typically considered absolute time between pesticide application and seedling planting. Seedlings in cold storage are not exposed to the elements of rain, wind, sunlight, or heat, and are typically stored at near or sub-zero temperatures. These conditions do not promote degradation of the pesticide residue on the seedlings. Information regarding cold storage of seedlings after pesticide application should be appropriately added to the application data that are included on pesticide application sheets that accompany each seedlot to a planting site.

CUMULATIVE EFFECTS

A possible cumulative effect from low level exposure to pesticide during tree planting was investigated using serial measurement of AChE and PChE activity during a tree planting season, as well as post-season measurement of ulnar and median motor nerve conduction velocity to define work-related biochemical and neurological adverse changes. Cholinesterase data were viewed using a cumulative sum and a best least-squares-fit logarithmic regression of serial AChE and PChE activity measures.

The possible existence of a non-zero time series trend for AChE and PChE activity during a tree planting season may be observed in the CUSUM values of Figures 2 and 3. Random fluctuation of enzyme activity about a constant mean value would result in a

CUSUM plot that oscillates around zero. This is not the case in any of the CUSUM plots presented. The AChE CUSUM value progressively increases, while the PChE CUSUM value progressively decreases during the planting season. The increasing AChE CUSUM value is surprising considering that organophosphate or carbamate exposure is expected to produce a decrease in AChE activity, and that exercise has been shown to produce an increase in PChE activity but to have no influence on erythrocyte AChE activity (Pawlowska *et. al.*, 1985b). Burgess and Roberts (1980) also observed an increase in the AChE CUSUM value after a worker's initial organophosphate exposure, however, it declined with subsequent exposures. These authors were unable to explain the rise in AChE, and stated that changes in PChE activity more closely reflected a change in a worker's daily protocol.

The PChE CUSUM value clearly shows a consistent decline in activity during the planting season, which is supported by the high frequency of individuals (77%) showing some inhibition of PChE during a work day. The finding is especially significant since the confounding effects of exercise on PChE activity would be expected to mask any inhibitory response to an anticholinesterase pesticide.

The relative importance of the rising trend in AChE activity is questionable based on the best fit logarithmic regression correlation coefficient and the small value of the slope coefficient (0.07) in the regression equation. Only a minor level of significance ($0.01 \leq p \leq 0.05$) could be assigned to the prework trend, while the postwork AChE trend showed a large degree of scatter and a regression of AChE against time was not definable. The prework and postwork PChE trends, on the other hand, showed a higher level of significance ($p \leq 0.01$) indicating that the observed logarithmic regression adequately represented the data. The fact that a logarithmic regression could be fit to the data leads to three important conclusions. First, the most rapid change in PChE activity is expected during the first week or two of tree planting. Second, the time to reach a significant depression of PChE activity from the baseline level may be estimated. Third, that this type

of relationship is representative of many biological processes reflecting a multicompartment, absorption, metabolic, and catabolic dose-response effect.

Expanding on the first conclusion, it has previously been reported that the appearance of anticholinesterase symptoms is more related to the rate of decline in cholinesterase activity than to the absolute level of activity (Trundle and Marcial, 1988; Aas *et. al.*, 1987; Coye *et. al.*, 1987; Sumerford *et. al.*, 1953). Thus a greater correlation between the change in cholinesterase activity and the appearance of symptoms should exist during an initial period of exposure to a detrimental pesticide. A logical approach to minimizing this effect would be to provide a "break-in" period at the beginning of a tree planter's season. Several steps could be taken to reduce a worker's pesticide exposure dose and optimize recovery time during the first few weeks of planting, the easiest method of which is to implement a shorter work day and work week, thus increasing the rest period between work weeks. An additional step requiring more extensive reorganization of the work would be to provide seedlings during the early season that have a lower level of pesticide residue. This could be achieved either by careful planning at the level of the nursery, or by better timing and organization of planting contracts throughout the season. If a contractor were made aware of the pesticide application history for each seedlot in advance of the planting season, it might be possible to arrange contracts such that the seedlings planted in the first month had the lowest level of pesticide residue. These steps may reduce the number of health complaints in the early season and therefore minimize the loss of newly recruited tree planters.

The estimated time to significant PChE inhibition, calculated from the regression equations derived for prework and postwork activity, is 130 days for prework PChE to reach 20% depression of the first day prework activity and 95 days for postwork PChE to reach 20% depression of the first day postwork activity. Restructuring the work-rest cycle to provide more recovery and a shorter duration of exposure would considerably extend these times. The typical tree planting season lasts between three and four months and is

therefore only marginally long enough to observe such an effect. On-going policy in B.C. Nurseries to reduce the use of pesticide on tree seedlings during growth may also be expected to prolong the interval required for a tree planter to reach a 20% cumulative inhibition of PChE.

It should be noted that Ratner *et. al.* (1989) observed a lower PChE activity in rural Israelis during summer compared with winter. These investigators attributed the decline in activity to an increased consumption of fresh fruit and vegetables during the summer. It is unlikely, however, that the trend observed in tree planters is due to a similar seasonal variation since there was a rapid decline in PChE activity that corresponded with the start of the tree planting season. The incorporation of an appropriate control group into the research design of this study (for example residents of nearby towns) would have provided a means of proving or disproving seasonal variation as a significant contributor to the observed trend.

Serum AChE Activity Increase

Assuming that the rise in AChE activity during the tree planting season was a "real" effect, it is possible that this increase was due to the induction of AChE in developing erythrocytes. That low-level chronic organophosphate or carbamate exposure can influence hematopoietic or hepatic mechanisms is supported by the variety of blood dyscrasias described in the literature (David and Fairchild, 1980; Davignon *et. al.*, 1965; Jegier, 1964). Similar influence could conceivably induce an increase in erythrocyte AChE synthesis during hematopoiesis.

Neurological Tests of Dysfunction

The cumulative effect of exposure to various pesticides during a tree planting season could also result in a decreased motor nerve conduction velocity and an increased distal latency, since this has been found in other individuals exposed to organophosphate or carbamate pesticide (Prinsen and Van Sittert, 1980; Ring *et. al.*, 1985; Wadia *et. al.*, 1987;

Misra *et. al.*, 1988). In this thesis, however, the group mean conduction velocity of the elbow to wrist segment for the ulnar and median nerves in both arms showed no significant improvement between an end of season and a recovery measurement several months later. In fact, the median nerve of the tree handling arm showed a slowing of conduction velocity and shorter distal latency that exceeded the standard error of the mean after a recovery period (see Figure 6). A paired t-test could not be applied to these data to search for statistically significant differences, due to the small sample size. This was due to the large rate of attrition in the post-season cohort. Seven of the initial 13 subjects were unavailable for retesting after several months of recovery. Three of the seven had left the country for extended vacations, two had moved with no forwarding address, and two chose not to return messages at their residence.

The scatter plot of Figure 7 illustrates the individual variation between end of season and recovery measures. The most obvious change in conduction velocity occurred in the shovel arm, where four of the six subjects showed an increase in velocity of the elbow to wrist segment of both the ulnar nerve and median nerve after recovery. This result is probably an indication of a reversible effect on the shovel handling forearm from the repetitive strain and/or impact of manipulating the shovel to open a hole for the planting of a seedling. Subject PE demonstrated the greatest slowing of conduction velocity in the tree handling arm, and also demonstrated an increase in conduction velocity for the shovel arm. This finding will also reflect the individual's off-season activity as a percussionist. The most marked slowing of conduction after recovery occured in the median nerve of the shovel arm in subject JR. No known explanation for this can be ascribed, apart from experimental error.

CHOLINESTERASE AND REPORTED SYMPTOMS

The four most frequent health complaints were joint swelling, fatigue, back pain, and muscle weakness, which comprised about 50% of the total number of complaints. This

indicates that the physical demands of tree planting and the repetitive nature of the work could probably account for the vast majority of symptoms reported. Nevertheless, there was a positive correlation between the number of general complaints and the degree of PChE inhibition, and between the number of non-zero general complaints and the degree of WChE inhibition. Many of the general symptoms that were reported, such as coughing, nose and eye irritation, skin rash, sore throat, headache, and dizziness are classically associated with pesticide exposure (Hodgeson and Parkinson, 1985; Midtling *et. al.*, 1985; Morgan, 1980; Milby, 1971; Sumerford *et. al.*, 1953). While this does not necessarily prove cause and effect, it is an indication that exposure to anticholinesterase pesticide could account for a significant portion of the general health complaints, which comprised 18% of the total number of complaints.

When the HSUM values were correlated to cholinesterase effect for each day of testing, it was found that a strong negative correlation existed between the total HSUM and each of AChE, PChE, and WChE prework-postwork percent change on May 11. This finding was supported by a consistently negative correlation between each of the categorized HSUMs and the cholinesterase measures on May 11. A significant negative correlation was also found between vagomimetic HSUM and WChE effect on June 10. All other significant correlations on other days were positive. The negative correlation indicates that fewer symptoms are reported as inhibition of cholinesterase increases. This is contrary to the expected result that the number of symptoms should increase in number and severity as the cholinesterase activity is progressively depressed by pesticide absorption. While a physiological explanation of these results is difficult, it is not a particularly surprising finding given the small sample size on the day indicated above and the typically small depression of cholinesterase activity. When the data for May 11 and June 10 are viewed on a scatter-plot, it is evident that only two individuals reported any symptoms on May 11 and that the HSUM for these individuals was only equal to one. On June 10, only one individual reported a non-zero HSUM which was again only equal to one. There was no

remarkable difference between individuals in the number of symptoms reported on these two days. Since relatively few tree planters demonstrated clinically significant inhibition of cholinesterase during the season, it is likely that the majority of reported symptoms were related to some other aspect of the work.

It is therefore logical to analyse further the relationship between the number of health complaints and the degree of cholinesterase inhibition in those tree planters that who demonstrated a significant depression of cholinesterase. Since there were very few individuals that showed a significant PChE inhibition, only the AChE data were analysed in this manner. Two individuals who showed significant AChE depression were excluded from this analysis due to the absence of their health questionnaire data on the relevant days. When the data for the remaining eleven individuals was processed, no significant correlation was found between any of the variables. It was noted upon viewing the scattergrams for each of the HSUM parameters against percent change in AChE activity that the two individuals who showed the most inhibition seemed to be significant outliers from the trend in the rest of the data (see Figures 6 and 7). When these individuals were removed from the database, there was a significant correlation of both total HSUM (r^2=0.588, n=9, $p \leq 0.01$) and musculoskeletal HSUM (r^2=0.520, n=9, $p \leq 0.01$) with the percent change in AChE activity. There was no statistically significant correlation of any other categorized HSUM values with AChE effect under these conditions.

Overall, it appears that there is an association between the total number of health complaints and the degree of change in AChE activity for those individuals that demonstrate significant inhibition of AChE. The association between reported symptoms and low level cholinesterase inhibition is not clear, and varies from day to day between a negative and positive correlation, although is rarely of statistical significance. Several factors may contribute to the absence of a clear relationship between reported symptoms and low level cholinesterase inhibition. Most obvious is the contribution of other aspects of tree planting work, such as the physical demand of the work, repetitive strain, shovel impact, and the

relative isolation of camp-life. The development of symptomatic tolerance to anticholinesterase effects has been well documented for chronic low-level exposure to organophosphate and carbamate chemicals. This may play a role in decreasing the incidence of symptoms related to pesticide absorption, and therefore in decreasing the possibility of finding a correlation between health complaints and percent change in cholinesterase activity, particularly for lower level cholinesterase inhibition. Thirdly, it has been found that the occurrence of anticholinesterase symptoms is more related to the rate of change in cholinesterase activity than to it's absolute level of activity (Trundle and Marcial, 1988; Aas *et. al.*, 1987; Coye *et. al.*, 1987; Sumerford *et. al.*, 1953). Except for a few rare incidents, the inhibition of cholinesterase was not remarkably large, indicating that the rate of change in cholinesterase activity was probably not rapid. It is also likely that an observed inhibition of cholinesterase activity was due to the prolonged low level exposure to pesticide during the eight to ten hours of tree planting on a given day, and not due to a single acute exposure to a high level of pesticide. The prolonged low level exposure will not result in the same rapid decline in cholinesterase activity that would be observed after acute high level exposure, and is therefore less likely to produce symptoms.

That general complaints could be positively correlated with the percent change in PChE activity for the entire cohort, while musculoskeletal complaints were positively correlated with the percent change in AChE activity only in significantly affected individuals is an interesting finding. It is possible that the effect of chronic low level pesticide absorption is more likely to result in a general peripheral syndrome, while higher level absorption is required in order to affect musculoskeletal functioning significantly. Musculoskeletal symptoms that are found to correlate with cholinesterase depression would then be expected to occur only in individuals with greater cholinesterase depression, while the general symptoms such as eye, nose, and skin irritation may occur at lower level cholinesterase depression. This is a logical progression of symptoms since many of the general symptoms relate directly to a person's initial contact with a pesticide, while the

musculoskeletal, psychological, and vagomimetic symptoms require absorption of the pesticide, its transport to tissue, interaction of the pesticide with the biochemistry of the tissue in question, and finally its degradation. The process of transportation and interaction would result in some degradation of the absorbed compound, particularly during the so-called 'first pass' through the hepatic circulation, as well as dilution of the absorbed dose in the body fluids. The pesticide dose that is actually available to interact with an individual's biochemistry and physiology may therefore be considerably smaller than the dose measured at the skin and external mucous membranes.

CONCLUSIONS

The relatively high incidence of significant acute AChE or PChE inhibition and the developing chronic inhibition of PChE during a tree planting season suggest that, although small, a toxicological hazard to tree planters exists from exposure to pesticide residue on the tree seedlings that they plant.

A relationship was established between the reported number of general health complaints and the degree of PChE and WChE inhibition. Since general health complaints comprised 18% of the total symptoms reported, it would not be unreasonable to expect a decrease in the number of reported health complaints if a tree planter's pesticide exposure was reduced. The vast majority of symptoms observed in this study, however, are more likely related to other aspects of tree planting work. Nevertheless, it is in the best interests of both the industry and the tree planter to try to minimize the discomfort and risk associated with reforestation. Reducing the effect of pesticide exposure on tree planters is one possible avenue. An understanding of the factors that contribute to significant pesticide absorption by a tree planter would be beneficial in successfully achieving this goal.

Although none of the measured exposure parameters could be positively correlated with inhibition of cholinesterase activity, an association was found between the carbamate reentry interval (CBRE) and inhibition of AChE activity, as well as the time from

organophosphate pesticide application until cold storage of the seedlings and inhibition of PChE activity. This suggests that the incidence of significant cholinesterase depression might be reduced by increasing the time between carbamate pesticide application and planting of seedlings, and/or by increasing the time between organophosphate pesticide application and cold storage of seedlings.

Although not significant overall, productivity was found to be significantly correlated with cholinesterase inhibition on several days of testing. It is difficult to apply this information meaningfully to aid in reduction of pesticide absorption in a practical way since the correlations were inconsistent, being sometimes positively correlated and other times negatively correlated. This suggests that although productivity may play a role in the absorption of pesticide, other related factors such as physical exertion and level of tree planting experience also contribute to the overall effect with a differing influence depending on the conditions of the day.

Finally, no consistent trend was noted in the neurophysiological measures. This was not unexpected given the small sample size and the low incidence of significant cholinesterase inhibition among the tree planters monitored.

The fact that there were some significant findings relating to the negative effect of pesticides in the results of this study suggests that further research and monitoring is warranted in the area of chronic occupational exposure to low level pesticide residue by reforestation workers. Several suggestions have been provided which are likely to reduce the incidence of significant pesticide absorption by a tree planter, some of which are easily implemented and some of which would require considerable restructuring of the silviculture industry. While the latter are unlikely to occur in the near future, it would be both cost effective and prudent to invoke the more easily implemented interventions suggested in this thesis in the interest both of protecting the individual tree planter's health and in producing a more stable, productive reforestation work force.

APPENDIX A
HISTORY QUESTIONNAIRE

NAME: SEX:

AGE: HEIGHT: WEIGHT:

PHONE NUMBER:

ADDRESS:

CITY: PROVINCE:

POSTAL CODE:

S.I.N.

HOW MANY YEARS HAVE YOU BEEN TREEPLANTING?

WHAT JOBS HAVE YOU HELD OVER THE PAST FOUR MONTHS?

HAVE ANY OF THESE JOBS INVOLVED CONTACT WITH PESTICIDES?

DO YOU HAVE ANY CURRENT OR CHRONIC MEDICAL CONDITIONS?

ARE YOU ON ANY MEDICATION?

IF YOU SMOKE, HOW MANY CIGARETTES/DAY?

IF YOU DRINK, HOW MANY DRINKS/DAY?
OR DRINKS /WEEK?

IF YOU USE 'ILLICIT' DRUGS, WHAT?
HOW OFTEN?

ARE YOU VEGETARIAN OR ON A RESTRICTED DIET OR USE DIET SUPPLEMENTS?

IF SO, PLEASE DESCRIBE:

APPENDIX B
WORK QUESTIONNAIRE

NAME:							
DATES:							
NUMBER OF TREES PLANTED							
SEEDLING LOT NUMBER							
NUMBER OF TREES PLANTED							
SEEDLING LOT NUMBER							
TIME TO SLEEP LAST NIGHT							
TIME AWAKE THIS MORNING							
TIME OF FIRST PLANTING RUN							
TIME OF LAST PLANTING RUN							
TIME SHOWERED/BATHED							
TIME CHANGED CLOTHES							
NUMBER OF BREAKS							
TOTAL BREAK TIME TODAY							
BUG REPELLANT WORN							
SUNTAN LOTION WORN							
CLOTHING WORN:**							
PANTS							
SHIRT							
JACKET/SWEATER							
HAT							
SCARF							
RAINGEAR							
GLOVES							
PLEASE DESCRIBE GLOVE TYPE:							

**PLEASE USE THE FOLLOWING CODES FOR CLOTHING WORN DURING TREEPLANTING:

W= WORN, NEWLY WASHED *X=WORN, NOT WASHED SINCE LAST WORN FOR PLANTING*

L= LONG SLEEVED/LEGGED *S=SHORT SLEEVED/LEGGED*

C=COTTON *P=POLYESTER* *O=OTHER MATERIAL*

EXAMPLE: PANTS W/L/C= NEWLY WASHED, LONG COTTON PANTS

PLEASE LIST ANY OTHER TREE HANDLING JOBS BESIDES PLANTING THAT YOU WERE INVOLVED IN , THE APPROXIMATE AMOUNT OF TIME THAT THIS INVOLVED, AND THE DAYS INVOLVED :
FOR EXAMPLE: LOADING SEEDLINGS INTO TRUCK, 1.5 HOURS ON JUNE 4, 5 AND 6.

APPENDIX C
HEALTH QUESTIONNAIRE

NAME:							
WEEK OF:							
HAVE YOU EXPERIENCED ANY OF THE FOLLOWING TODAY? PLEASE INDICATE WITH AN X	DATE						
ABDOMINAL PAIN							
BACK PAIN							
BLURRED VISION							
CONSTIPATION							
COUGHING							
DEPRESSION							
DIARRHEA							
DIZZINESS							
EYE IRRITATION							
FATIGUE							
HEADACHE							
INSOMNIA							
IRRITABILITY							
JOINT SWELLING OR PAIN							
LOSS OF APPETITE							
MUSCLE CRAMPING							
MUSCLE TWITCHING							
MUSCLE WEAKNESS							
NAUSEA							
NIGHT SWEATING							
NOSE BLEED							
NOSE IRRITATION							
NOSE RUNNING							
PALPITATIONS/ALTERED HEART RHYTHM							
SKIN IRRITATION OR RASH							
SORE THROAT							

ANY ADDITIONAL COMMENTS OR SYMPTOMS TO REPORT?
(FOR EXAMPLE: IF YOU CHECKED "SKIN IRRITATION" ABOVE, WHAT AREA OF YOUR BODY?)

ISSUED ON:
03/23/89

MINISTRY OF FORESTS - SILVICULTURE
HEAD5101: PESTICIDE APPLICATION DETAIL REPORT

SORTED BY REG, DIST, AGENCY, PLYS, SEEDLOT

REGION: N - DISTRICT: 05 - AGENCY: WESTAR - PLANTING Y/S: 895

SEEDLT	REQKEY	SPECIES	STOCK TYPE	REQ AGE	PESTICIDE APPLIED	FORMUL DESC	PRODUCT/LT OF SOLN	RATE AI/HA	APPLIC DATE	REASON	METHOD
00848	88N05855	FDI	PSB313A	100000	IPRODIONE	50WP	1SGR	1.5	881201	STORAGE	SPRAYER
05320	87N05825	FDI	BBR	200000	SIMAZINE	FLOW.LIQ	.002L/L.	.5L	880414	WEEDS	BOOM SPYR
					SEVIN	WET PWDR	1 GM	.5 GM	880418	BUDWORMS	BOOM SPRAY
					PERMETHRIN (AMBUSH)	EMUL.CON	.129 ML	70 ML/HA	880603	CRANBERRY GRID.	BOOM SPRY
					PERMETHRIN	EMUL.CON	.129 ML	70ML/HA	880623	CUTWORMS	BOOM SPRY
					PERMETHRIN	EMUL.CON	0.129 ML	70 ML/HA	880714	CRAN. GIRDLER	BOOM SPRAY
05461	87N05820	SE	PSB415B	200000	DIAZINON	50 EC	1.36 ML/L	914.6ML/HA	880614	APHIDS	MONKOFF
					DIAZINON	50EC	1.36ML	914.6ML	880614	APHIDS	MONKOFF
					BENLATE	50WP	.63GR	420GR	881123	PRE-STORAGE	MONKOFF
					CAPTAN	50WP	1.87GR	838GR	881123	PRE-STORAGE	MONKOFF
	87N05821	SE	PSB415B	200000	DIAZINON	50 EC	1.36 ML/L	914.6ML/HA	880614	APHIDS	MONKOFF
					DIAZINON	50EC	1.36ML	914.6ML	880614	APHIDS	MONKOFF
					BENLATE	50WP	.63GR	420GR	881123	PRE-STORAGE	MONKOFF
					CAPTAN	50WP	1.87GR	838GR	881123	PRE-STORAGE	MONKOFF
05651	87N05823	SE	PSB313B	200000	SEVIN	85 WP	W/ERAN	N/A	870702	CUT WORMS	BAIT
					ORTHENE	75	0.026 GM	638 GM	870807	CRAN. GIRDLER	INJECTION
05726	88N05842	PLI	PSB313A	100000	BRAVO	40.4	1.25ML	0.606L	881007	BOTRYTIS	MONKOFF
					BENLATE	50WP	93GR	420GR	881123	PRE-STORAGE	MONKOFF
					CAPTAN	50WP	1.87GR	838GR	881123	PRE-STORAGE	MONKOFF

REFERENCES

Aas, P., Veiteberg, T.A., Fonnum, F. (1987) Acute and sub-acute inhalation of an organophosphate induce alteration of cholinergic muscarinic receptors. *Biochemical Pharmacology, 36* (8), 1261-1266.

Abiola, F.A., Sere, A., Sawadogo, J.G., Diatta, F., Ly, M. (1988) Cholinesterase depression among Senegalese crop protection workers exposed to organophosphorus pesticides. *Bulletin of Environmental Contamination and Toxicology, 41*, 483-488.

Apol, A.G., Thoburn, T.W. (1976a) Health hazard evaluation determination report no. HHE-75-THIRAM-352, Composite report on Thiram. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

Apol, A.G., Thoburn, T.W. (1976b) Health hazard evaluation determination, composite report on Thiram for HHE-76-188-353, Challenge Reforestation, Inc.. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

Apol, A.G., Thoburn, T.W. (1976c) Health hazard evaluation determination, composite report on Thiram for HHE-75-137-354, The Hoedads. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

Apol, A.G., Thoburn, T.W. (1976d) Health hazard evaluation determination, composite report on Thiram for HHE-75-184-356, Klamath Reforestation, Inc.. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

Apol, A.G., Thoburn, T.W. (1976e) Health hazard evaluation determination, composite report on Thiram for HHE-75-140-357, Webfoot Reforestation. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

Apol, A.G., Thoburn, T.W. (1976f) Health hazard evaluation determination, composite report on Thiram for HHE-75-123-358, Weyerhauser Company, Centralia, Washington. NIOSH, Hazard evaluation services branch, Cincinnati, Ohio.

B.C. Research (1986) Exposure of Tree Seedling Nursery Employees to Captan, Benomyl and Chlorothalonil. B.C. Research project number 3-15-162; FIIHRP project number F&L85-2.

B.C. Research (1988). Exposure of Treeplanters to Fungicides. B.C. Research project number 4-02-194; FIIHRP project number FL/85-1.

Banister, E.W., Robinson, D.G., and Trites, D.G. (1990) Ergonomics of Treeplanting. Forestry Canada and the British Columbia Ministry of Forests, FRDA Report 127.

Berends, F., Posthumus, C.H. (1959) The chemical basis of the aging process of DFP-inhibited pseudocholinesterase. *Biochimica et Biophysica Acta, 34*, 576-.

Brimijoin, S. (1986). Modern approaches to the biology of cholinesterases. *Federation Proceedings, 45*, 2958-2959.

Brown, S.K., Ames, R.G., and Mengle, D.C. (1989) Occupational illnesses from cholinesterase-inhibiting pesticides among agricultural applicators in California, 1982-1985. *Archives of Environmental Health, 44(1)*, 34-39.

Bull, D. (1982) A growing problem: pesticides and the third world poor. Oxford: OXFAM, 38. Cited in: Magnotti, R.A., Dowling, K., Eberly, J.P., McConnell, R.S. (1988) Field measurement of plasma and erythrocyte cholinesterases. *Clinica Chimica Acta, 315*, 315-332.

Burgess, J.E., Roberts, D.V. (1980) A longitudinal study of red cell and plasma cholinesterase in two groups of organophosphorus workers. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 99-103.

Callaway, S., Davies, D.R., Rutland, J.P. (1951) Blood cholinesterase levels and range of personal variation in a healthy adult population. *British Medical Journal*, 2,812-816.

Cessna, A.J. (1988) Pesticide Safety. Agriculture Canada Publication 1825E; Cat No A43-1825/1988E.

Chambers, H.W., Chambers, J.E. (1989) An investigation of acetylcholinesterase inhibition and aging and choline acetyltransferase activity following a high level acute exposure to paraoxon. *Pesticide Biochemistry and Physiology, 33*, 125-131.

Copplestone, J.F. (1980) Methods for field assessment of exposure to pesticides. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 17-19.

Corrao, G., Calleri, M., Carle, F., Russo, R., Bosia, S., Piccioni, P. (1989) Cancer risk in a cohort of licensed pesticide users. *Scandinavian Journal of Work Environment and Health, 15(3)*, 203-209.

Costa, L.G. (1988) Interactions of neurotoxicants with neurotransmitter systems. *Toxicology, 49*, 359-366.

Coye, M.J., Barnett, P.G., Midtling, J.E., Velasco, A.R., Romero, P., Clements, C.L., Rose, T.G. (1987) Clinical confirmation of organophosphate poisoning by serial cholinesterase analyses. *Archives of Internal Medicine, 147*, 438-442.

Cozby, P.C. (1981) *Methods in behavioural research.* Mayfield Publishing, Palo Alto, California.

Dar Lin, S. (1985) Giardia lamblia and water supply. *J A.W.W.A.: Management and Operations*, February, 40-47.

Das Gupta, S., Bass, K.N., Warnick, J.E. (1989) Interaction of reversible and irreversible cholinesterase inhibitors on the monosynaptic reflex in neonatal rats. *Toxicology and Applied Pharmacology, 99*, 28-36.

David, A., Fairchild, E.J. (1980) Occupational pesticide hazards in the activities of WHO's office of occupational health: Hematological criteria for health assessment. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 3-15.

Davignon, L.F., St.-Pierre, J., Charest, G., Tourangeau, F.J. (1965) *Canadian Medical Association Journal, 92(12)*, 597-602.

Davis, T.M. (1981) *Caution before yield: pesticides and tree planters.*

Dedek, W. (1980) Solubility factors affecting pesticide penetration through skin and protective clothing. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 47-50.

Derache, R. (Ed.) (1977) *Organophosphorus Pesticides: Criteria (Dose/Effect Relationships) for Organophosphorus Pesticides. Report of a Working Group of Experts prepared for the Commission of the European Communities, Directorate-General for Social Affairs, Health and Safety Directorate.* Pergamon Press, Toronto.

Dille, J.R., Smith, P.W. (1964) Central nervous system effects of chronic exposure to organophosphate insecticides. *Aerospace Medicine, 35*, 475-478.

Drenth, H.J., Engsberg, I., Roberts, D., Wilson, A. (1972) Neuromuscular function in agricultural workers using pesticides. *Archives of Environmental Health, 25*, 395-398.

Duell, P.B., Morton, W.E. (1987) Henoch-Schonlein purpura following Thiram exposure. *Archives of Internal Medicine, 147(4)*, 778-779.

Duncan, R.C., Griffith, J., Konefal, J. (1986) Comparison of plasma cholinesterase depression among workers occupationally exposed to organophosphorus pesticides as reported by various studies. *Journal of Toxicology and Environmental Health, 18*, 1-11.

Ellman, G.L., Courtney, K.L., Andres V.Jr., Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemistry and Pharmacology, 7*, 88-95.

Engel, A.G., Lambert, E.H., Santa, T. (1973) Study of long-term anticholinesterase therapy: effects on neuromuscular transmission and on motor end-plate fine structure. *Neurology, 23*, 1273-1281.

Fenichel, G.M., Kibler, W.B., Olson, W.H., Dettbarn, W. (1972) Chronic inhibition of cholinesterase as a cause of myopathy. *Neurology, 22*, 1026-1033.

Ganong, W.F. (1983) *Review of Medical Physiology.* Lange Medical Publications, Los Altos, California.

Goulding, R. (1988) Accidental pesticide poisoning: the toll is high. *World Health Forum, 9*, 526-530.

Gray, P.J., Duggleby, R.G. (1989) Analysis of kinetic data for irreversible enzyme inhibition. *Biochemical Journal, 257*, 419-424.

Gunther, F.A., Iwata, Y., Carman, G.E., Smith, C.A. (1977) The citrus reentry problem: research on its causes and effects, and approaches to its minimization. *Residue Reviews, 67(1).*

Hammond, P.S., Forster, J.S. (1989) A microassay-based procedure for measuring low levels of toxic organophosphate compounds through acetylcholinesterase inhibition. *Analytical Biochemistry, 180*, 380-383.

Hodgeson, M.J., Parkinson, D.K. (1985) Diagnosis of organophosphate intoxication. *New England Journal of Medicine, 313 (5)*, 329.

Hussain, M.A., Oloffs, P.C., Blatherwick, F.J., Gaunce, A.P., MacKenzie, C.J.G. (1981) Detection of incipient effect of anticholinesterase insecticides in rats and humans by electromyography and cholinesterase assay. *Journal of Environmental Science and Health, B16(1)*, 1-19.

Hussain, M., Yoshida, K., Atiemo, M., Johnston, D. (1990) Occupational exposure of grain farmers to carbofuran. *Archives of Environmental Contamination and Toxicology, 19*, 197-204.

Iwata, Y., Knaak, J.B., Spear, R.C., Foster, R.J. (1977) Worker reentry into pesticide-treated crops. I. Procedure for the determination of dislodgeable pesticide residues on foliage. *Bulletin of Environmental Contamination and Toxicology, 18*, 649-655.

Jager, K.W, Roberts, D.V., Wilson, A. (1970) Neuromuscular function in pesticide workers. *British Journal of Industrial Medicine, 27*, 273-278.

Jegier, Z. (1964) Health hazards in insecticide spraying of crops. *Archives of Environmental Health, 8*, 670-674.

Karalliedde, L., Senanayake, N. (1988) Acute organophosphorus poisoning in Sri Lanka. *Forensic Science International, 36*, 97-100.

Katz, L.S., Marquis, J.K. (1989) Modulation of central muscarinic receptor binding *in vitro* by ultralow levels of the organophosphate paraoxon. *Toxicology and Applied Pharmacology, 101*, 114-123.

Kilpatrick, J.S. (1973) *Statistical Principles in Health Care Information.* University Park Press, Baltimore, Maryland, 1973.

Knaak, J.B. (1980) Minimizing occupational exposure to pesticides: techniques for establishing safe levels of foliar residues. *Residue Reviews, 75*, 81-96.

La Du, B.N., Lockridge, O. (1986) Molecular biology of human serum cholinesterase. *Federation Proceedings, 45*, 2965-2969.

Lamb, D.W. (1980) Early studies with azinphos methyl to determine re-entry times for citrus pickers. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 121-127.

Lockridge, O., Eckerson, H.W., La Du, B.N. (1979) Interchain disulphide bonds and subunit organization in human serum cholinesterase. *Journal of Biology and Chemistry, 254*, 8324-8330.

Loosli, R. (1980) Monitoring program for the assessment of human safety in operations involving organophosphorus insecticides. In: *Field worker exposure during*

pesticide application. Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 93-98.

Lotti, M., Becker, C., Aminoff, M. (1984) Organophosphate polyneuropathy: pathogenesis and prevention. *Neurology, 34*, 658-62.

Ma, D.M., Liveson, J.A. (1983) *Nerve Conduction Handbook.* F.A. Davis Company, Philadelphia.

Magnotti, R.A., Dowling, K., Eberly, J.P., McConnell, R.S. (1988) Field measurement of plasma and erythrocyte cholinesterases. *Clinica Chimica Acta, 315*, 315-332.

McDonald, E., Chung, L., Higgs, A. (1988) Safe clothing for pesticide use. Workers' Compensation Board of B.C., *Health and Safety Digest, 23(4).*

McMaster, S.B., Finger, A.V. (1989) Effects of exercise on behavioural sensitivity to carbamate cholinesterase inhibitors. *Pharmacology Biochemistry and Behaviour, 33*, 811-813.

Melchers, B., Van Helden, H. (1990) On the development of behavioural tolerance to organophosphates II: neurophysiological aspects. *Pharmacology Biochemistry and Behaviour, 35*, 321-325.

Midtling, J.E., Barnett, P.G., Coye, M.J., Velasco, A.R. Romero, P., Clements, C.L., O'Malley, M.A., Tobin, M.W., Rose, T.G., Monosson, I.H. (1985) Clinical management of field worker organophosphate poisoning. *Western Journal of Medicine, 142*, 514-518.

Milby, T.H., Ottoboni, F., Mitchell, H.W. (1964) Parathion residue poisoning among orchard workers. *Journal of the American Medical Association, 189 (5)*, 351-356.

Milby, T.H. (1971) Prevention and management of organophosphate poisoning. *Journal of the American Medical Association, 216 (13)*, 2131-2133.

Misra, U.K., Nag, D., Khan, W.A., Ray, P.K. (1988) A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. *Archives of Toxicology, 61*, 496-500.

Morgan, D.P. (1980) Minimizing occupational exposure to pesticides: acute and chronic effects of pesticides on human health. *Residue Reviews, 75*, 97-102.

Ngatia, J., Mgeni, A.Y. (1980) The effects of continuous exposure to organophosphorus and carbamate insecticides on cholinesterase (CHE) levels in humans. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 63-66.

Nursery 1987: *Production Guide for Commercial Growers.* Province of British Columbia, Ministry of Agriculture and Fisheries.

Olson, C.B., Swett, C.P.Jr. (1971) Effect of prior activity on properties of different types of motor units. *Journal of Neurophysiology, 34*, 1-16.

Pawlowska, D., Moniuszko-Jakoniuk, J., Soltys, M. (1985a) The effect of chronic physical exercise on the activity of hydrolytic enzymes in acute poisoning with

parathion-methyl in rats. *Polish Journal of Pharmacology and Pharmacy, 37*, 639-646.

Pawlowska, D., Moniuszko-Jakoniuk, J., Soltys, M. (1985b) Parathion-methyl effect on the activity of hydrolytic enzymes after single physical exercise in rats. *Polish Journal of Pharmacology and Pharmacy, 37*, 629-638.

Peiris, J.B., Fernando, R., De Abrew, K. (1988) Respiratory failure from severe organophosphate toxicity due to absorption through the skin. *Forensic Science International, 36*, 251-253.

Popendorf, W.J., Leffingwell, J.T. (1982) Regulating OP pesticide residues for farmworker protection. *Residue Reviews, 82*, 125-201.

Popendorf, W.J. (1985) Advances in the unified field model for reentry hazards. In: *Dermal Exposure Related to Pesticide Use: Discussion of Risk Assessment..* Honeycutt, R.C., Zweig, G., Ragsdale, N.N. (Eds.), American Chemical Society, Washington, pp 323-340.

Prinsen, G.H., Van Sittert, N.J. (1980) Exposure and medical monitoring study of a new synthetic pyrethroid after one season of spraying on cotton in Ivory Coast. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 105-120.

Quillfeldt, J., Raskovsky, S., Dalmaz, C., Dias, M., Huang, C., Netto, C.A., Schneider, F., Izquierdo, I., Medina, J.H., Silveira, R., Dajas, F. (1990) Bilateral injection of fasciculin into the amygdala of rats: effects on two avoidance tasks, acetylcholinesterase activity, and cholinergic muscarinic receptors. *Pharmacology Biochemistry and Behaviour, 37*, 439-444.

Raffaelle, K., Hughey, D., Wenk, G., Olton, D., Modrow, H., McDonough, J. (1987) Long-term behavioural changes in rats following organophosphoronate exposure. *Pharmacology Biochemistry and Behaviour, 27*, 407-412.

Raheel, M. (1987) Resistance of selected textiles to pesticide penetration and degradation. *Journal of Environmental Health, 49(4)*, 214-219.

Ratner, D., Bar Sella, P., Schneeyour, A., Kardontchik, A., Eshel, E. (1989) Seasonal variation in blood cholinesterase activity. *Israel Journal of Medical Science, 25*, 247-250.

Raveh, L., Ashani, Y., Levy, D., De La Hoz, K., Wolfe, A.D., Doctor, B.P. (1989) Acetylcholinesterase prophylaxis against organophosphate poisoning: Quantitative correlation between protection and blood-enzyme level in mice. *Biochemical Pharmacology, 38(3)*, 529-534.

Richter, E.D., Gribetz, B., Krasna, M., Gordon, M. (1980) Heat stress in aerial pilots. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 129-136.

Ring, H., Melamed, S., Heller, L., Solzi, P. (1985) Evaluation of EMG examination as an indicator of worker susceptibility to organophosphates exposure. *Electromyography and Clinical Neurophysiology, 25*, 35-44.

Roberts, D.V., Wilson, A. (1969) Electromyography in diagnosis and treatment. In: *Myasthenia gravis*. Greene, R. (Ed.), Heinemann Publishing, London, 29-42.

Roberts, D.V. (1980) Blood cholinesterase monitoring of workers exposed to organophosphate pesticides: theory and practise. In: *Field worker exposure during pesticide application*. Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 173-179.

Rosenberry, T.L., Roberts, W.L., Haas, R. (1986) Glycolipid membrane-binding domain of human erythrocyte acetylcholinesterase. *Federation Proceedings, 45*, 2970-2975.

Ryhanen, R., Hanninen, O. (1987) A simple method for the measurement of blood cholinesterase activities under field conditions. *General Pharmacology, 18(2)*, 189-191.

Ryhanen, R., Kajovaara, M., Harri, M., Kaliste-Korhonen, E., Hanninen, O. (1988) Physical exercise affects cholinesterases and organophosphate response. *General Pharmacology, 19(6)*, 815-818.

Schumacher, M., Camp, S, Maulet, Y., Newton, M., MacPhee-Quigley, K., Taylor, S.S., Friedman, T., Taylor, P. (1986) Primary structure of acetylcholinesterase: implications for regulation and function. *Federation Proceedings, 45*, 2976-2981.

Screef (1989) Letter from Pierre Richard: Planters sick from sprayed trees. 6(March/April), p7.

Senanayake, N., Johnson, M.K. (1982) Acute polyneuropathy after poisoning by a new organophosphate insecticide. *New England Journal of Medicine, 306 (3)*, 155-157.

Senanayake, N., Karalliedde, L. (1987) Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. *New England Journal of Medicine, 316(13)*, 761-763.

Serat, W.F. (1973) Calculation of a safe reentry time into an orchard treated with a pesticide chemical which produces a measureable physiological response. *Archives of Environmental Contamination and Toxicology, 1 (2)*, 170-181.

Shih, J.H., Wu, Z.Q., Wang, Y.L., Zhang, Y.X., Xue, S.Z., Gu, X.Q. (1985) Prevention of acute parathion and Demeton poisoning in farmers around Shanghai. *Scandinavian Journal of Work and Environmental Health, 11*, 49-54.

Silver, A. (1974) *The Biology of Cholinesterases*. Frontiers of Biology, Volume 36. Eds: Neuberger, A., and Tatum, E.L.. American Elsevier Publishing Company, New York.

Singh, A.K. (1985) Kinetic analysis of inhibition of brain and red blood cell acetylcholinesterase and plasma cholinesterase by acephate or methamidophos. *Toxicology and Applied Pharmacology, 81*, 302-309.

Smith, T.J. (1987) Occupational characteristics of tree planting work. *Silviculture Magazine, Jan/Feb*, 12-17.

Spear, R.C., Jenkins, D.L., Milby, H. (1975) Pesticide residues and field workers. *Environmental Science and Technology, 9 (4)*, 308-313.

Speight, B. (1980) Effects of formulation upon the safe use of pesticides. In: *Field worker exposure during pesticide application.* Tordoir, W.F., van Heemstra, E.A.H.(Eds.). Elsevier, New York, 29-37.

Spencer, W.F., Iwata, Y., Kilgore, W.W., Knaak, J.B. (1977) Worker reentry into pesticide-treated crops. II. Procedures for the determination of pesticide residues on the soil surface. *Bulletin of Environmental Contamination and Toxicology, 18*, 656-662.

Standaert, F.G. (1964) The mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. *Journal of General Physiology, 47*, 987-1001.

Sumerford, W.T., Hayes Jr., W.J., Johnston, J.M., Walker, K., Spillane, J. (1953) Cholinesterase response and symptomatology from exposure to organic phosphorus insecticides. *Archives of Industrial Hygiene and Occupational Health, 7*, 383-398.

Takahashi, H., Kato, A., Yamashita, E., Naito, Y., Tsuda, S., Shirasu, Y. (1987) Potentiations of N-methylcarbamate toxicities by organophosphorus insecticides in male mice. *Fundamental and Applied Toxicology, 8*, 139-146.

Tordoir, W.F., van Heemstra, E.A.H.(Eds.) (1980) *Field worker exposure during pesticide application: Proceedings of the fifth international workshop of the Scientific Committee on Pesticides of the International Association on Occupational Health*, The Hague, The Netherlands, October 9-11, 1979. Elsevier Publishing, New York, 187-188.

Trundle, D., Marcial, G. (1988) Detection of cholinesterase inhibition: the significance of cholinesterase measurements. *Annals of Clinical Laboratory Science, 18(5)*, 345-353.

Van Dongen, C., Wolthius, O. (1989) On the development of behavioural tolerance to organophosphates I: behavioural and biochemical aspects. *Pharmacology Biochemistry and Behaviour, 34*, 473-481.

Vancouver Sun (1981) Chemical history should be on every box of trees. *June 2*, p5, Pacific Press.

Vancouver Sun (1986) Diseases felling planters. *July 19*, pp C1/C7, Pacific Press.

Vandekar, M. (1980) Minimizing occupational exposure to pesticides: cholinesterase determination and organophosphorus poisoning. *Residue Reviews, 75*, 67-80.

Veronesi, B., Jones, K., Pope, C. (1990) The neurotoxicity of subchronic acetylcholinesterase (AChE) inhibition in rat hippocampus. *Toxicology and Applied Pharmacology, 104*, 440-456.

Voss, G., Sachsse, K. (1970) Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylthiocholine/DTNB procedure. *Toxicology and Applied Pharmacology, 16*, 764-772.

Wadia, R.S., Chitra, S., Amin, R.B., Kiwalkar, R.S., Sardesai, H.V. (1987) Electrophysiological studies in acute organophosphate poisoning. *Journal of Neurology Neurosurgery and Psychiatry, 50*, 1442-1448.

Wecker, L., Kiauta, T., Dettbarn, W. (1978) Relationship between acetylcholinesterase inhibition and the development of a myopathy. *Journal of Pharmacology and Experimental Therapeutics, 206 (1)*, 97-104.

Wicker, G.W., Williams, W.A., Bradley, J.R. Jr., Guthrie, F.E. (1979) Exposure of field workers to organophosphorus insecticides: cotton. *Archives of Environmental Contamination and Toxicology, 8*, 433-440.

Williams, M.W., Cook, J.W., Blake, J.R., Jorgensen, P.S., Frawley, J.P. (1958) The effect of parathion on human red blood cell and plasma cholinesterase. *Archives of Industrial Health, 18*, 441-445.

Wolfsie, J.H., Winter, G.D. (1952) Statistical analysis of normal human red blood cell and plasma cholinesterase activity values. *Archives of Industrial Hygiene and Occupational Health, 6*, 43-49.

World Health Organization (1975) Pesticide residues in food. Report of the 1974 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies No. 97, WHO Technical Report No. 574. Cited in: Derache (1977) p106.

Xue, S.Z. (1987) Health effects of pesticides: a review of epidemiologic research from the perspective of developing nations. *American Journal of Industrial Medicine, 12*, 269-279.

Younkin, S.G., Goodridge, B., Katz, J., Lockett, G., Nafziger, D., Usiak, M.F., Younkin, L.H. (1986) Molecular forms of acetylcholinesterases in Alzheimer's disease. *Federation Proceedings, 45*, 2982-2988.

Zubay, G.(Ed.) (1983) *Biochemistry*. Addison-Wesley Publishing Company, Don Mills, Ontario, 154-156.

Zweig, G., Gao, R., Witt, J.M., Popendorf, W.J., Bogen, K.T. (1985) Exposure of strawberry harvesters to Carbaryl. In: *Dermal Exposure Related to Pesticide Use: Discussion of Risk Assessment*. Honeycutt, R.C., Zweig, G., Ragsdale, N.N. (Eds.), American Chemical Society, Washington, pp 123-138.