CHOLINESTERASE STATUS OF SOME ETHIOPIAN PEST CONTROL WORKERS EXPOSED TO ORGANOPHOSPHATE PESTICIDES

A THESIS PRESENTED TO THE

SCHOOL OF GRADUATE STUDIES

ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN BIOLOGY

BY

KIBRUYISFA LAKEW

JUNE, 1996

ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my research advisor Dr. Yalemtsehay Mekonnen, for her close supervision, constructive guidance and encouragement throughout the course of this investigation.

I owe a special dept of gratitude to the pest control workers of the Upper Awash Agro-industry Enterprise, Northern Omo, Wollega state farms and members of the region 14 labour and social affairs bureau, who voluntered to be experimental subjects. I am also thankful to the managers , adminstrators and clinic staffs of the aforementioned state farms for their sincere co-operation.
I would like to express my deep appreciation to Dr. Endeshaw Bekele for sending me reprinted articles from abroad and Ato Temesgen Zewotir and Ato Yirsaw Ayalew who sacrificed their time in making the statistical analysis.

Thanks are due to Ato Hailu Tegegnework, Ato Seyoum Amelmal and Ato Hailu Lema of the National Research Institute of Health, for their co-operation in collecting the blood samples.

Appreciation is also extended to departmental colleagues and my friends who have kindly helped me in many ways. I am particularly greatful to Ato Asrat Bekele and Ato Zerihun Gezahegne for their unreserved support in the preparation of this thesis.

I am also greatiful to the Sweden agency for research co-operation in developing countries (SAREC) for the financial assistance that made this project possible.

Finally, I wish to appreciate my dearest brothers Dr. Tenaye Lakew, Ato Habtamu Lakew and my dearest friends, w/t Aster Tsegaye and w/t Ethiopia Mekuria without whose constant support and encouragement this thesis could not have been possible.

TABLE OF CONTENTS

CONTENT PAGE

LIST OF TABLES

LIST OF PICTURES

A B B R E V I A T I O N S

ACh	Acetyl choline
AChE	Acetylcholinesterase
Ap	Alkaline phosphatase
BuChE	Butyrylcholinesterase
ChE	Choilinesterase
ESCAP	Economic and Social Commission of Asia and the Pasific
GPT	Glutamic pyruvic transaminase
IDRC	International Development Research Center
IFCC	International Federation for Clinical Chemistry
ILO	International Labour Organization
N.Omo	Northern Omo
NTE	Neuropathy target esterase
OIDP	Organophosphorus-induced delayed polyneuropathy
OC	Organochlorine
OP	Organophosphate
PChE	Pseudocholinesterase
SCE	Scandinavian Committee on Enzymes
UAAE	Upper Awash Agro-industry Enterprise
UNDP	United Nations Development Programme

A B S T R A C T

A study was conducted in 430 pest control workers with that of 161 comparable controls. The health risks and associated morbidity from occupational exposure to *organophosphate (OP)* pesticides was determined and factors that may contribute to a greater pesticide exposure were identified.

Plasma and RBC *cholinesterase (ChE)* activities were determined using electrometric methods. Symptoms of *OP* poisoning and risk factors for exposure associated with pesticide use were assessed through a questionnaire, developed based on the WHO (1982) and WHO-UNDP (Maroni et al 1986) recommendations. To evaluate the possible liver dysfunction, plasma levels of *alkaline phosphatase (AP)* and *glutamic pyruvic transaminase (GPT)* were determined using diagnostic kits number 6391 and 14442520 respectively.

Significantly lower plasma and RBC *ChE* activities were found in the exposed workers compared to the controls ($P<0.001$); 58 (13.5%) and 47 (10.9%) of the exposed workers had plasma and RBC *ChE* activities below the lower limit of the controls respectively. Compared to the workers in the other job categories, the "spray men" and those with duration of exposure below 5 years were identified to be the most affected groups.

Normal *AP* and *GPT* levels without significant differencee from the controls were noted, suggesting the absence of any marked liver impairemnet at this level of exposure. Symptoms of *OP* poisoning were frequent in the exposed workers than in the controls. However, except for sweating and weakness ($p<0.05$), other symptoms reported were not significantly associated with low RBC and plasma *ChE* activities. Surveys of the risk factors for possible pesticide poisoning revealed that lack of knowledge and poor personal hygiene were prominent among the exposed population

In general, the present study showed that there are apparent health risks associated with *OP* exposure. Measures that should be considered to minimize the health effects from the use of *OP* pesticides are recommended; such as pre-placenment and periodic medical examinations, the use of appropriate protective devices and creation of awareness among the workers of the risks of pesticide poisoning.

1. INTRODUCTION

Pesticides have made a very significant contribution towards increasing the quality and quantity of crop protection world wide, as well as putting under control scourges which once caused untold death and famine in many countries (ILO, 1979). Some 15,000 individual compounds and more than 35,000 formulations have come into use as pesticides since 1945 (Forget, 1991-a).

Despite these advantages, most pesticides are inherently toxic to all living things, a fact that users are learning the hard way. Since pesticides are poison by design (Forget, 1991 -a), they constitute a potential risk to the producer, the user, the bystander and the consumer (White, 1993). Some are persistent in the environment, bioaccumulate in fatty tissues and cause long_term chronic problems, nervous system disorders, and sterility (Akhabuhaya, 1991). Therefore, concern has grown during the past years as to their potentially harmful effects to man and the environment.

Several surveys conducted by the World Health Organization (WHO) and others concerned painted a progressively alarming picture. This was summarized by Foo (1985): In 1977, based on notifications from several governments and surveys of nine countries, WHO estimated that the number of deaths globally was about 24,640 a year. In 1981 OXFAM updating the WHO figures estimated that world pesticide related poisonings were around 750,000 a year. More recently, the economic and social commission of Asia and the pacific (ESCAP) suggested that pesticide poisoning incidents might amount to two million a year, of which 40,000 could be fatalities.

The situation is much worse in developing countries, where illiteracy, poverty and general morbidity rates are high (Mawanthi and Kimani, 1993). It is estimated that 375,000 severe cases occur annually, with 10,000 deaths, this corresponds to about one case per minute and one death per hour (Aguilar et al., 1993).

Pesticides consist primarily of *organochlorine (OC)*, *organophosphate (OP)* and *carbamate* compounds (Wu et al., 1989). *Organochlorine* pesticides are generally less inherently toxic than *OP* pesticides, although their neurotoxic action can also affect non_target organisms. However, their danger lies with their relative resistance to environmental degradation which allows them to accumulate in the upper part of the food pyramid (Forget, 1991-b; Forget 1993). Because of these reasons, the production of *OP* and *carbamate* compounds has grown rapidly to replace organochlorinated hydrocarbons which degrade more slowly and have a higher environmental impact (Brown et al ., 1978; Sansur et al., 1993). Therefore, these two classes of pesticides account for the majority of pesticides registered worldwide (Mineau and Keith, 1993). Although these groups of pesticides present less danger to the ecology than chlorinated hydrocarbons, the risk of human toxicity associated with their use is far greater (Spigel et al., 1981). Many of them are highly toxic and are especially responsible for more cases of poisoning than all other pesticides (Plestina, 1984). *Organophosphates* were introduced first as "nerve gases" during World War II. Because of their non-specificity and non-selectivity among animal life, these compounds pose a great danger of toxicity to humans, accidentally and/or deliberately (Popendorf and Leffingwel, 1982).

Organophosphates and *carbamates* are neuroactive molecules which act by disrupting the recovery mechanism of cholinergic neuronal transmission (ILO, 1979; Spigel et al., 1981; Coye et al., 1986). They inhibit the enzyme *cholinesterase (ChE)* which transmits nerve impulses to nerve or effector cells in the body (ILO, 1979). Since all earth multicellular animals rely on some

form of cholinergic system, these compounds are extremely toxic to non-target organisms, including humans (Namba, 1971; Forget, 1991-b).

Pesticides are absorbed through all possible routes, including the skin, the lungs, the gastrointestinal tract, and the conjunctiva; they may also enter the body by injection although this is of rare occurrence (Namba, 1971). The circumstances of exposure (duration, formulation, task performed, weather conditions), malnutrition, diseases and fatigue influence the absorption of toxic ingredients and actions of these materials on vital tissues (ILO, 1979).

Agricultural workers are at particularly high risk for pesticide-related illnesses. By job category, these are primarily the mixers, loaders and applicators who handle agricultural chemicals directly (Coye et al., 1986; Ciesielski et al., 1994).

Inspite of the magnitude and severity of the health and environmental problems associated with pesticides, these chemicals will continue to be widely used in the foreseeable future both in agriculture and in public health (Mercier, 1993). On the other hand, the use of these chemicals for economic benefit has generally preceded recognition of risk or harmful outcomes (Forget, 1993).

Reports of *OP* toxicity and fatal human poisonings associated with the use of these agents first appeared in the literature following world war II (Namba, 1971; Spigel et al., 1981). Since these initial reports, acute and chronic *OP* pesticide poisoning has been described in crop workers, in commercial pesticide applicators, in children and suicide attempts by adults (Spigel et al., 1981).

In many areas of the world today, there is still a paucity of information on the prevalence of poisonings by pesticides. The situation is much worse, particularly for the countries of the

developing world (Jeyaratnam, 1993). Although the hazards arising from pesticides appear to be more serious in the developing countries than in the developed countries, research that has been carried out in developing countries is inadequate. The nature, magnitude and severity of the problem are not known with certainty. In developing countries where about 99 percent of pesticide-related poisonings occur, few studies have been conducted because of financial and technical constraints (Amr et al., 1993). Moreover, there are only a few reports of the health effects of occupational exposure to *OP* pesticides (Misra et al., 1985).

Ethiopia is a country covering a vast area with wide spread agricultural activity. The national economy is dependent on agriculture and this sector uses enormous quantities of pesticides to improve crop yields. The use of these chemicals in homes to combat household pests, disease vectors and grain pests is also becoming increasingly common.

In Ethiopia, the use of pesticides in the agricultural sector is wide spread and intensive. For example, in 1979/80, it was known that about 37,281 liters of 18 different brands of pesticides were spread in the Awash valley state farms, and over 0.7 million liters of insecticides were used in the same farms in a 6 year period against cotton pests (Abegaz, 1983). Reports have been made that misapplication of pesticides have been implicated in the finding of pesticides in the tissue of agricultural labourers (Zharov et al, 1981). Except for such kind of scanty information, no epidemiologic study have been carried out to findout the extent of pesticides poisoning among the agricultural workers (Asmerom, 1995). The acute and chronic health effects from the use of *OP* pesticides have not been described. Generally, no documented information about the types of pesticides in use and their possible effects on man and the environment exists in the country. It is important, therefore, that epidemiological studies of the health impacts of pesticides on humans be carried out in Ethiopia.

In pursuance of this objective, this study was undertaken among agricultural workers in three major state owned farms in the country. Namely, the Upper Awash Agro-industry Enterprise (UAAE), Wollega and Northern Omo (N.Omo) state farms.

In the present study the health effects of *OP* pesticides on the agricultural workers were evaluated based on medical histories, measurements of *ChE* levels and liver function tests. The findings were analysed in relation to age, job category, duration of exposure and causal factors of exposure that are related to pesticide use.

The specific objectives of this study are:

1. To assess the *ChE* and liver function status of pest control workers exposed to *OP* pesticides.,
2. To compare *ChE* activities of the exposed workers among the different study sites, job categories and duration of exposure and identify the high risk groups.,
3. To determine the relationship between *ChE* activities and prevalence of *OP* poisoning signs and symptoms.,
4. To identify risk factors that may contribute to a greater Pesticide exposure.

2. LITERATURE REVIEW

2.1. The Enzyme *Cholinesterase*.

There are two main types of *cholinesterases: acetylcholinesterase* (*AChE*, specific *ChE*, true *ChE*, acetylhydrolase of *acetyl choline* E C 3.1.1.7) and a less specific enzyme (called *pseudocholinesterase, butyrylcholinesterase* (*BuChE*, acylhydrolase of acylchline, E C 3.1.1.8)) (Namba, 1971; Bacq et al., 1971; Abiola et al., 1988). There are distinguishing characteristics between *AChE* and *PChE*. *Aetylcholinesterase* is found in the central nervous system, motor endplates of skeletal muscle and in human erythrocytes (Namba, 1971) and placenta (Kangas and Jauhiainen, 1991). *Pseudocholinesterase* is found in human plasma (serum), liver (Namba, 1971; Bacq et al., 1971; Abiola et al., 1988), adipocyte and smooth muscle (Abiola et al., 1988).

These esterases are differentiated by (1) substrate specificity, (*AChE* hydrolyses acetyl-β-methylcholine but very little benzoylcholine, propionylcholine, or butyrylcholine, while the opposite is true for *PChE*); (2) selective inhibition; and (3) substrate inhibition (*AChE* is inhibited by *acetylcholine* concentrations equal to or greater than 4 mM and *PChE* by concentrations greater than 100 mM) (Namba, 1971).

Acetylcholinesterase plays a great role for hydrolysing *acetylcholine* (*ACh*) into choline and acetic acid on neural influx transmission in the cholinergic system. The non-synaptic *AChE* is postulated to maintain excitability and to initiate and propagate action potentials in nerve and muscle by regulating passive and active transport of electrolytes (Bacq et al., 1971). The physiological function of *PChE* however, remains unknown (Kangas and Jauhiainen, 1991).

2.2. Factors affecting *Cholinesterase* activity.

Blood *ChE* activity is variable both at individual and group levels. Age, sex and race influence *ChE* activity (Kangas and

Jauhiainen, 1991). The influence of age and sex on human *ChE* activity has been studied by many investigators. The reported findings however, were controversial. Shanor and his coworkers (1961) found no difference between the plasma *ChE* level of middle-aged and old individuals. Red Blood Cell *ChE* activity doesn't change with age in adults. Studies that show plasma *ChE* changes for both sexes with age are contradictory. However, even when reported, the differences with increasing age are very small (Quinones et al., 1976; Coye et al., 1986). There is no difference in RBC *ChE* activity associated with sex, whereas Plasma *ChE* activity is significantly higher in males than in females (Shanor et al., 1961). On the other hand, other investigators found no intersex difference in *PChE* or RBC *ChE* levels (Brown et al., 1978).

Race is also known to influence *ChE* activity. It is reported that plasma but not RBC *ChE* activity to be lower in black persons than in white persons of the same sex. However it is unknown whether this is the result of genetic or nutritional factors (Coye et al., 1986). Many diseases depress plasma *ChE* levels. The effects on serum *ChE* of many diseases which affect hepatic function are well known. Neoplasms, parenchymal liver disease, acute infections, and some aneamias all depress serum *ChE* values (Baetjer, 1983; Coye et al., 1986). Most of these conditions also prevent the individual from working. However, they are rarely responsible for depressed *ChE* in occupationally exposed populations (Coye et al., 1986). Studies also suggest that persons subject to nutritional deficiency and/or water deficit, as is frequently encountered in migrant and seasonal farm workers, could be the vulnerable groups (Baetjer, 1983; Forget, 1991-a).

A small number of workers exhibit an unusual sensitivity to some quaternary ammonium esterase inhibitors; such workers also have reduced serum *ChE* activity. The genetic variance is not associated with any increased susceptibility to *AChE* inhibition or to poisoning by *OPs* and *carbamates* (Coye et al., 1986). Pregnancy is also known to alter the concentration of many blood proteins including *ChE*. An acute drop in *ChE* values

was found in the first trimester with a slight apparent but nonsignificant rise in the third trimester, where as the lowest values were observed during the second to seventh days of postpartum (Howard, 1978).

2.3. Cholinergic Transmissions.

The cholinergic synapses, in which *ACh* is the transmitter include, synapses in the central nervous system, neuromuscular junctions, sensory nerve endings, ganglionic synapses of both sympathetic and parasympathetic nerves, postganglionic sympathetic nerve terminals that innervate the sweat glands and blood vessels, sympathetic nerve terminals (without ganglionic synapses) in the adrenal medulla, and all postganglionic parasympathetic nerve terminals (Namba, 1971; Bacq et al 1971). Under normal physiological conditions, at the cholinergic synapses, *ACh* is released from the nerve ending as the neurohumoral transmitter in response to the nerve impulse, and initiates excitation by reacting with the receptor. *Acetylcholine* is then hydrolysed by *AChE* into choline and acetylated *AChE*; the latter reacts with water, dissociating into acetic acid and active *AChE* (Namba, 1971; Bacq et al., 1971). This reaction is completed rapidly and the synapse becomes ready for the next impulse.

2.4. Physiology of *organophosphate* poisoning.

The molecular basis of the action of *OPs* on neural influx transmission has been well studied. It is directly related to inhibition of *AChE* within the central and peripheral nervous system, promoting the accumulation of *ACh* at the nerve synapse (Spigel et al., 1981). In cases of poisoning, *AChE* is bound to the *organophosphorus* compound, and its organic residue dissociates, leaving the phosphate group bound with the esteric site of *AChE* (Namba, 1971). This reaction produces a relativey stable and biologically inactive enzyme (Kangas and Jauhiainen, 1991). Since the rate at which this phosphorylated *AChE* dissociates is so slow (as to be practically negligible), *organophosphorus* compounds are said to be "irreversible"

inhibitors (ILO, 1979; Coye et al., 1986). As a result of *AChE* inhibition, *ACh* molecules accumulate at the synapse, initially causing excessive excitation and latter leading to the blockage of synaptic transmission. The inhibition of *AChE* may be considered to be an extremely slow enzymatic hydrolysis of the *organophosphorus* compound molecule (Namba, 1971).

Monitoring the effects of groups of compounds with specific methods well established for *OPs* is independent to their chemical structures. Usually, only the effect of the compounds on enzymes in the body fluids is measured. Therefore, depression of plasma and/or RBC *ChE* activity is the most satisfactory and generally available evidence of excessive absorption of *OP* pesticides (ILO, 1979). Blood *ChE* activity is only convenient indicator of the status of neural *AChE* (Popendorf and Leffingwel, 1982). The cholinergic effects of anti*ChE* compounds are due to the depression of the activity of the nervous system, muscle and secretary glands, and not of the plasma or RBCs directly. However, since it is not possible to determine the *ChE* activity of these tissues in man during life, it is necessary to rely on the *ChE* activity of the plasma and RBCs as a guide of some value in detecting absorption of the anti*ChE* compounds (Grob et al.,1950). It is a measure of the biochemical effect of the pesticide rather than a measure of the pesticide itself (Amr et al., 1993). The RBC *ChE* has been reported to closely parallel the level of *ChE* in the central and peripheral parasympathetic nervous system and is considered to be the best indicator of *AChE* activity at the nerve synapses (Namba, 1971). Although determination of plasma *ChE* is used because of its simplicity, the determination of both the RBC and the plasma *ChE* is preferable, because most *OP* pesticides preferentially inhibit either plasma or RBC *ChE*. In cases of long-term exposures, however, the RBC *ChE* value is more important (Kangas and Jauhiainen, 1991).

Monitoring acute intoxications by short-term over-exposures to *OPs*, is recommended to be carried out either in plasma or in whole blood. In the case of chronic or intermittent but relatively low exposure to *OPs*, the only reliable determination

is the assessment of *AChE* activity in red blood cells. Inhibition of this activity can only be correlated with the effects on the nervous system. After exposure to *OPs*, the recovery to pre-exposure values is much faster for plasma *ChE* than for RBC ChE. Recovery of *AChE* activity is parallel to the production of new erythrocytes and to the de novo synthesis of *AChE* in neurons. The relatively long recovery time for *AChE* makes its monitoring very suitable for measuring previous or repeated low level exposure to *OPs* (Lewalter and Korallus, 1986). Plasma *ChE* is completely regenerated in 50 days, while RBC *ChE* regenerates more slowly, at a rate of 1% or less per day. Red blood cells have a life of about 120 days, and hence the blood levels recover slowly (Rama, 1995).

2.5. Signs and Symptoms of *Organophosphate* Poisoning.

The chemical manifestations of exposure to *OP* compounds are extremely diverse. Major poisoning among exposed workers are fairly well characterized but, the health effects of routine, smaller exposures are uncertain (Ciesielski et al., 1994). The physical characteristics of the active material of the pesticide, its penetration ability, stability, distribution, persistence and degradation in the body considerably influence the likelihood of the appearance of outward effects in man (ILO, 1979; Plestina, 1984).

Organophosphates can be grouped into two categories: those which can act on *ChE* directly without being modified and those which first have to be transformed in the body and therefore are called indirect inhibitors (Plestina, 1984). For instance, the toxicity of malathion is due mainly to *ChE* inhibition following the conversion of the compound to malaxon in the liver (Namba, 1971). After over exposure to direct inhibitors, signs and symptoms usually appear fairly quickly following exposure. Indirect inhibitors produce a more delayed and prolonged action, and when Symptoms appear, although exposure might cease, they may develop further to cause a critical illness (Plestina, 1984).

In general, the acute cholinergic effects of severe *OP* poisoning correlate well with *ChE* inhibition. Chronic moderate exposure results in a cumulative inhibition of the RBC and plasma enzymes. The appearance of symptoms depends more upon the rate of fall in *ChE* activity than the absolute level of activity reached. Workers may exhibit 70% to 80% inhibition of both plasma and RBC *ChE* levels after several weeks of moderate exposure without manifesting cholinergic symptoms. On the other hand, a previously unexposed individual may develop symptoms after sudden exposure and a rapid drop in *ChE* activity of less than 30% (Coye et al., 1986). It is generally accepted that during repeated exposure to toxic doses of *OP* compounds, symptoms of poisoning occur when 50% of the RBC *ChE* is inhibited (Kangas and Jauhiainen, 1991) and when 50% of the plasma *ChE* is inhibited (Namba, 1971). A 40% decrease in blood *AChE* is associated with the first symptoms of pesticide poisoning; an 80% decrease is associated with severe neuromuscular effects. Absorption of *OPs* sufficient to inactivate all the red blood cell *AChE* may result in death (Sansur et al., 1993).

Workers exposed to *OP* pesticides were reported to exhibit a wide variety of symptoms: headache, visual blurring and vomiting with malathion; sweating , eye irritation, impairment of vision, cramps, salivation with fenthion; nausea, headache and nonspecific symptoms with parathion (Misra et al., 1985). Most of the manifestations of poisoning by *OP* compounds are associated with excessive cholinergic action. On the other hand, tachycardia and increased blood pressure are explained by overwhelming cholinergic effects on the central nervous system, sympathetic ganglionic synapses, or adrenal medulla (Namba, 1971; Coye et al., 1986). Symptoms commonly associated with cholinergic excess (nausea, dizziness, vomiting, abdominal pain, and ataxia) observed to appear early but, abate with in a month. Symptoms of headache, weakness, anorexia and blurred vision persist longer (Coye et al., 1986). In mild poisoning, the manifestations are predominately due to stimulation of parasympathetic nerve endings. Manifestations resulting from

the stimulation of other nerve endings appear in moderate or severe poisoning (Namba, 1971).

Local exposure to *OP* pesticides produce comparatively severe local manifestations. Exposure of the eye causes severe miosis and lachrymation. Dermal exposure may cause copious sweating. Absorption from the respiratory tract may cause tightness of the chest initially and dyspnoea and bronchial secretions later. The ingestion of *OP* compounds is often followed by severe abdominal pain, diarrhoea, and vomiting (ILO, 1979; Plestina 1984).

In recent years clinical effects that cannot easily be ascribed to the classic anti*ChE* inhibition of *OP* pesticides have emerged. These manifestations are distinct from the well defined cholinergic phase that characterizes the normal acute cases of poisoning, but also from delayed neuropathy, which has a much later onset (Forget, 1991-a). The syndrome are described as an *organophosphorus*-induced delayed polyneuropathy (OPIDP). It is related to the organophosphorylation of a protein in the nervous system called neuropathy target esterase (NTE) (Lotti, 1986). There is also a report that an intermediate *OP* poisoning syndrome, involving the paralysis of a number of muscle sets including the respiratory muscles, that has resulted in death of a number of patients (Forget, 1991-a). Persistent central nervous system manifestations such as impaired memory, depression, impaired mental concentration and instability are also reported. There has also been reports of abnormal liver function and histological abnormalities of the liver. Other possible persistent effects include changes in coagulation factor, effects on the fetus, dermatitis, bronchial asthma and impotence (Namba, 1971).

3. MATERIALS AND METHODS

3.1. Description of the study sites.

The study was conducted in three different state farms namely; the Northern Omo, the Wollega and the UAAE farms.

3.1.1. The Northern Omo (N.Omo) State Farm.

The N.Omo state farm is located about 550 kms south west of Addis Ababa. Previously it was under Awash agricultural development corporation. Since 1993 however, it was reorganized under independent enterprise. There are three main sub farms, named Abaya, Sile and Arba Minch, each about 115, 30 and 15 kms away from Arba Minch town respectively. The altitude is 1250 to 1300 meters above sea level. The average rain fall is 3.6 mm/annum with a mean minimum and maximum temperatures of 17.7 ^{0}c and 31.9 ^{0}c respectively (data from farm office). There are about 90 workers permanently involved in pest control activities. Agricultural activitiy in such farms is mainly rain-fed. Maize, cotton, banana and mango are the major products of the farm.

3.1.2. The Wollega State Farm.

The Wollega state farm is located about 400 kms north west of Addis Ababa. It covers a total area of 23,745 hectares with an average altitude of 1,440 meters above sea level. The mean rain fall is 4.4 mm/annum with a mean minimum and maximum temperatures of 13.8 and 30.0 ^{0}c respectively. There are nine sub farms namely, Lugo, Anger, Diddesa, Aukie, Kenaf, Horo Aleltu, Luko, Bereda, and Bello, each about 50 to 90 kms away from Nekemte. The total working population is about 13,443 out of which 2,410 are permanent employees. There are about 180 workers involved in pest control activities (data from farm office). The main products of this farm include maize, cotton, sourghum and sunflower. Similar to the Northern Omo farms, production is rain-fed and the major activities of the farm follow the rainy season.

3.1.3. The Upper Awash Agro-industry Enterprise (UAAE).

The Upper Awash Agro-industry farms were previously under the Awash agricultural development corporation. Since 1993 however, it was reorganized as an independent enterprise. It is located about 200 kms east of Addis Ababa. It covers a total area of about 6,500 hectares. The four major sub farms of this enterprise are Nura Era, Merti Jeju, Tibila and Awara Melka, each 20, 5, 55, and 110 kms away from the enterprise office located at Merti Jeju. The average altitude is 1018 (range 785-1170) meters above sea level. The mean rain fall is 4.04 mm/annum, with a mean minimum and maximum temperatures of 16.3 ^{0}c and 32.6 ^{0}c respectively. There are about 200 permanent workers involved in crop protection activities (data from farm office). Perhaps, this is the largest horticultural farm in the country. The main products being mango, melon, papaya, robbi beans, orange, lemon, tomato, pepper, teff, maize, and to a lesser extent cotton and tobacco.

Unlike the Northern Omo and Wollega farms, the UAAE farms involve extensive irrigation schemes using the Awash river as a source. Therefore, pesticide applications are practised throughout the year.

3.2. Pesticides in use.

The physical form of technical grade of *OP* pesticides used in the state farms studied varies from volatile liquids, through semi-liquids and waxes to crystalline solids.

Some of the *ChE*-inhibiting pesticides used in the study sites are given in Table 1. These pesticides are nearly known under proprietary or trade names. Therefore a multitude of these exist, often several for the same active ingredient in various formulations, concentrations or for mixtures of two or more active ingredients. Description of the toxicity category is based on the guidelines of classification of pesticides by hazard (WHO 1994). As it can be seen from table 1, almost all types of *OP* pesticides are used in the UAAE farms.

Most of these are applied as insecticides in all the farm fields except Detiagas and Quickphos which are mostly used as fumigants to combat insects in grain stores. Dursban, Guazatine, Volathion, Actalic, Ultracide, Dimecron and Malathion were *OPs* most frequently sprayed in the respective state farms.

Table 1. List of common anti*ChE* pesticides used in the state farms during the study period.

Pesticide common name	Toxicity category*	Farms using the Pesticide
Actalic	Moderately hazardous	UAAE + Wollega
Azodrin	Extremely hazardous	UAAE
Bazodin	--	UAAE + Wollega
Curacuron	Extremely hazardous	All farms
Detiagas	--	All farms
Diazinon	--	UAAE
Dimecron	Moderately hazardous	All farms
Dursban	Moderately hazardous	All farms
Ecathion	--	N.Omo
Guazatine	Highly hazardous	UAAE
Malathion	Slightly hazardous	UAAE + Wollega
Metasystox	Highly hazardous	N.Omo + UAAE
Nuvacron	Moderately hazardous	UAAE
Parathion	Extremely hazardous	UAAE
Quickphos	--	UAAE + Wollega
Rogor	Moderately hazardous	UAAE
Sevin	--	N.Omo
Sumithion	Highly hazardous	Wollega
Ultracide	--	UAAE
Volathion	Highly hazardous	UAAE + N.Omo

* According to the WHO (1994)

3.3 The study population.

A total population of 591 participated in this study. Volunteer non-exposed subjects, pest control workers and farm managers were briefly introduced about the aim of the study. Thus, they were encouraged and agreed to participate in the study. All measuments were carried out based on the consent of the subjects.

3.3.1 Exposed subjects

All pest control workers from the aforementioned study sites participated in the study. They were all Ethiopians working in pesticide-exposed fields during the study period. From the three study sites, a total of 430 workers (all male), took part in the study. No female was involved in pest control activities. Of these workers, 81 were from the N.Omo state farms; 169 were from the Wollega state farms; and 180 were from the UAAE farms.

All workers in the different occupational groups were considered separately as recommended by WHC protocols (1982). The main job categories considered were the "spray men" (mixers, loaders, applicators and flagmen), tractor operators, pest assessors and mechanics. The study was designed as much as possible not to interfere with the manner in which they are normally carried out in pest control operations such as tank filling, container rinsing, pesticide application, pest assessing and spray machine maintenance.

3.3.1.1 "Spray men" (mixers-loaders-applicators-flaggers).

"Spray men" consisted of persons who were directly involved in the preparation and application of pesticides. The mode of application could be aerial, ground or by backpack means. "Spray men" may apply pesticides by carrying manual or power-operated machines on their shoulders. They also apply pesticides using tractor-drawn air spray equipment, fitted with a pump and a spray gun. They spray under climatic conditions they considered appropriate. Mixers are workers who dilute

pesticide formulations and make ready for application. The kind of formulation used for mixing could be wettable powder, granular dust, emulsifiable concentrate or solvent soluble. Loaders load diluted formulations in the tankers of applicating machines. Loading could be into motorized knapsack spray equipment, tractor-drawn air spray machines, aeroplane tankers or other manual spray equipment. Flaggers are workers who guide the direction of pilots during aerial sprays. Using white flags hold by the flaggers, the pilots could be able to differentiate pesticide treated fields from the untreated ones. However, it is the usual practice in all the study sites that mixing, loading, flagging and spraying operations to be done by the same worker. Workers alternate between mixing, loading or spraying activities. Therefore, all workers in these job categories were considered as "spray men". Pictures 1 to 10 show some of these pesticide applications and related practices in the study areas.

3.3.1.2 Tractor Operators.

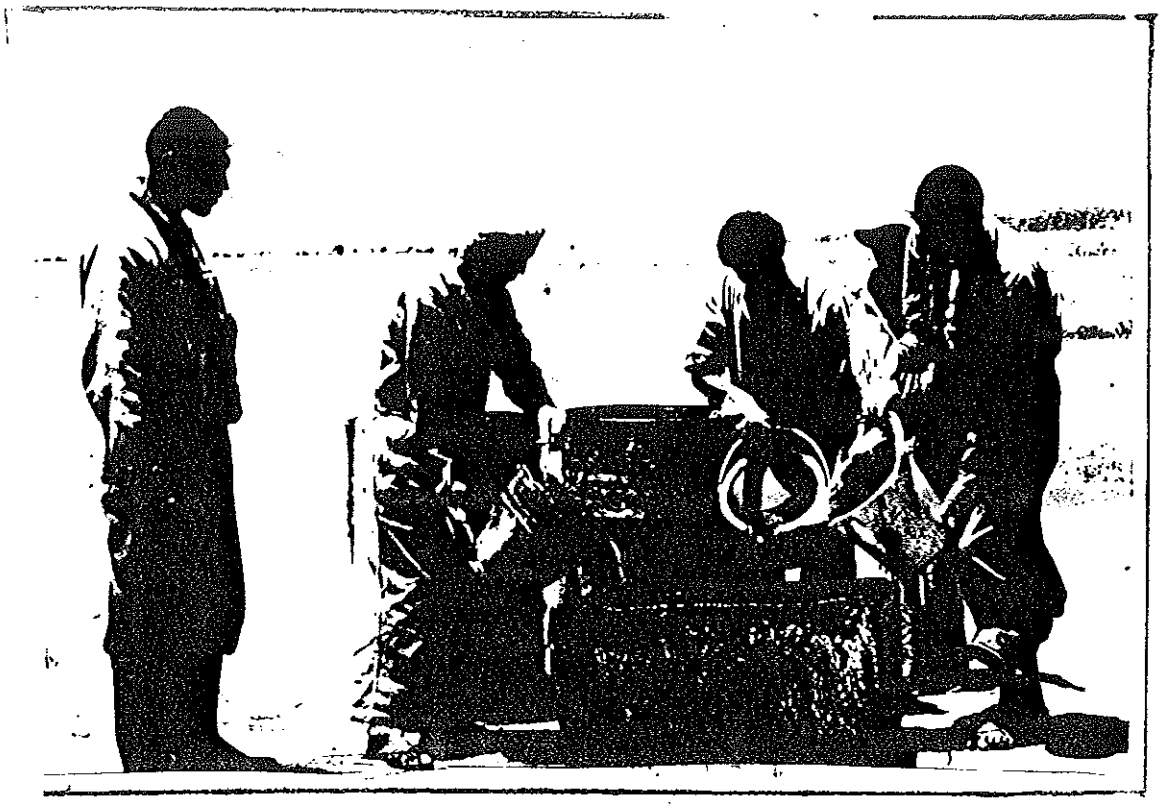
The tractor operators are workers who drive tractors loaded with pesticide applicating machines. During tractor-operated pesticide applications, they operate tractor-pulled ground rigs which consisted of trailer-mounted spray tanks equipped with booms at the rear and operated with pumps powered by tractor take-offs. During pesticide applications of large tree fruits, tractor operators help the "spray men" by moving tractors from treated to untreated rows.

3.3.1.3 Pest Assessors.

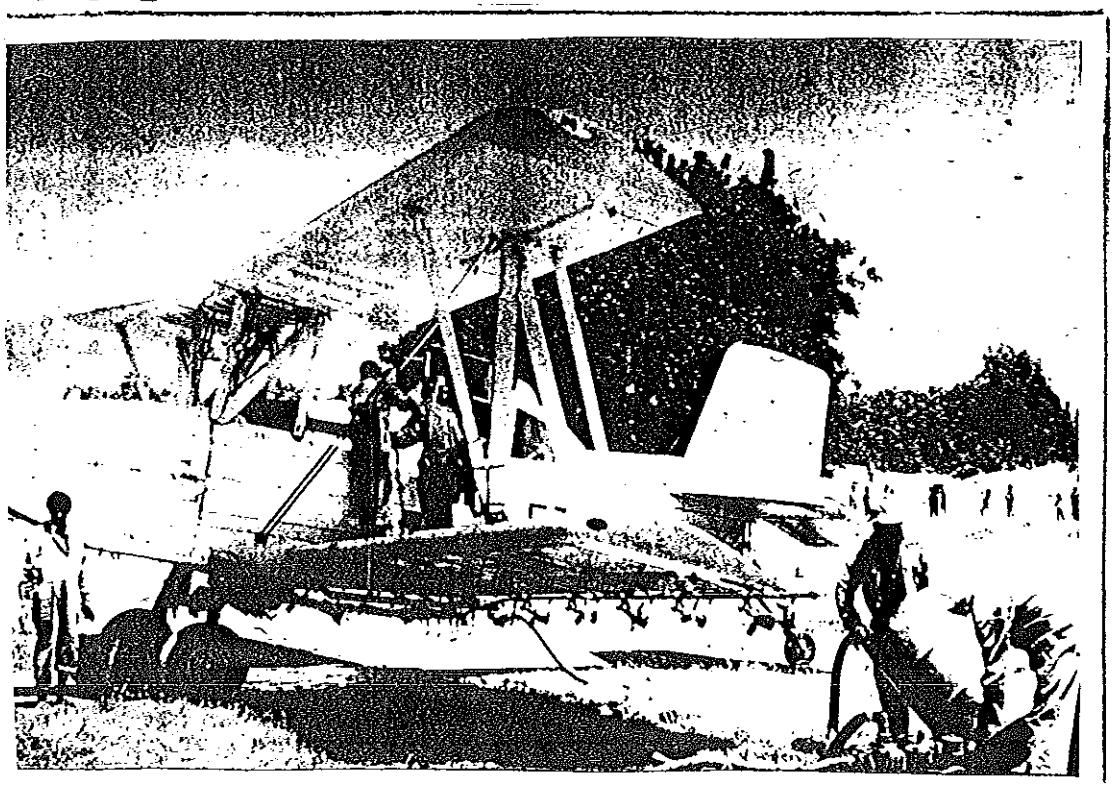
The pest assessors are leaf inspectors who assess the presence or absence of crop pests before and after pesticide treatment. Assessment of pesticide treated fields is commonly conducted after 24, 48, and 72 hours of pesticide application.

3.3.1.4 Mechanics.

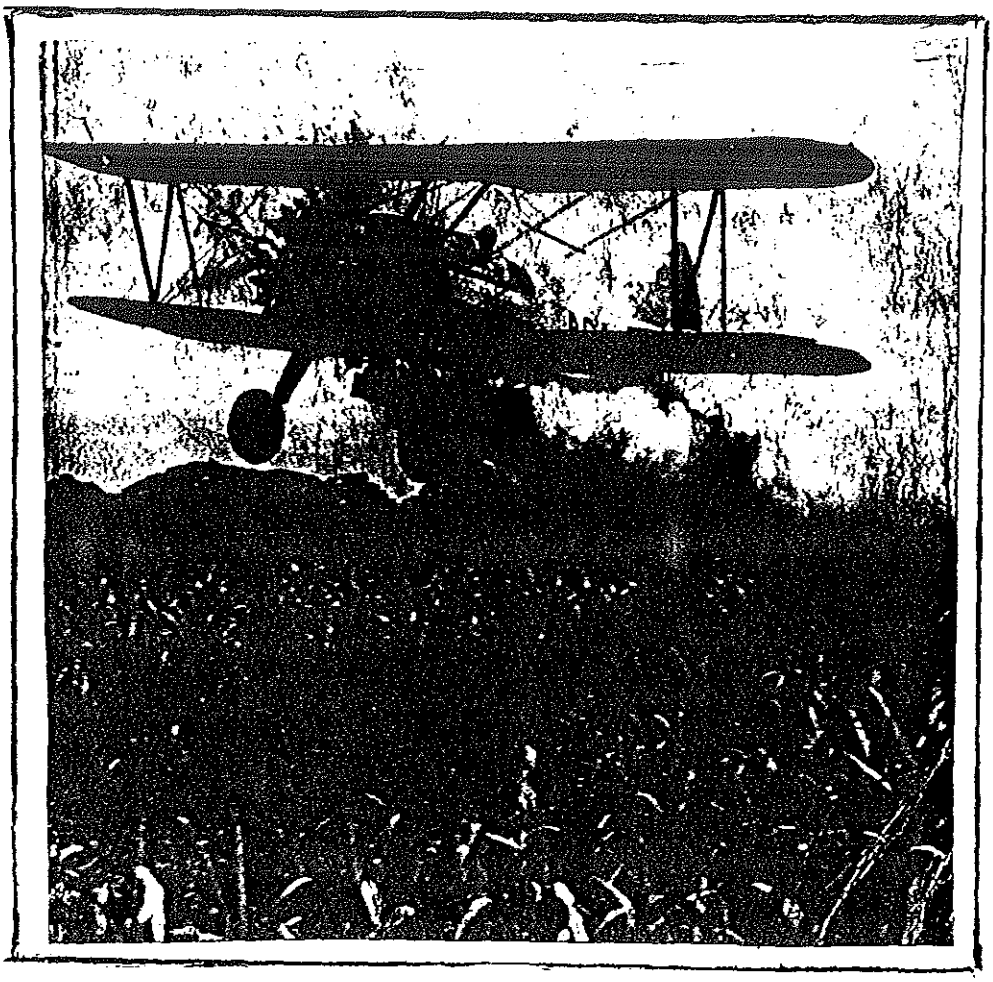
These are technicians servicing and cleaning spray machines, tankers, hoses and other equipment. They are directly or indirectly exposed to diluted pesticide formulations during maintenance of contaminated applicating equipment.



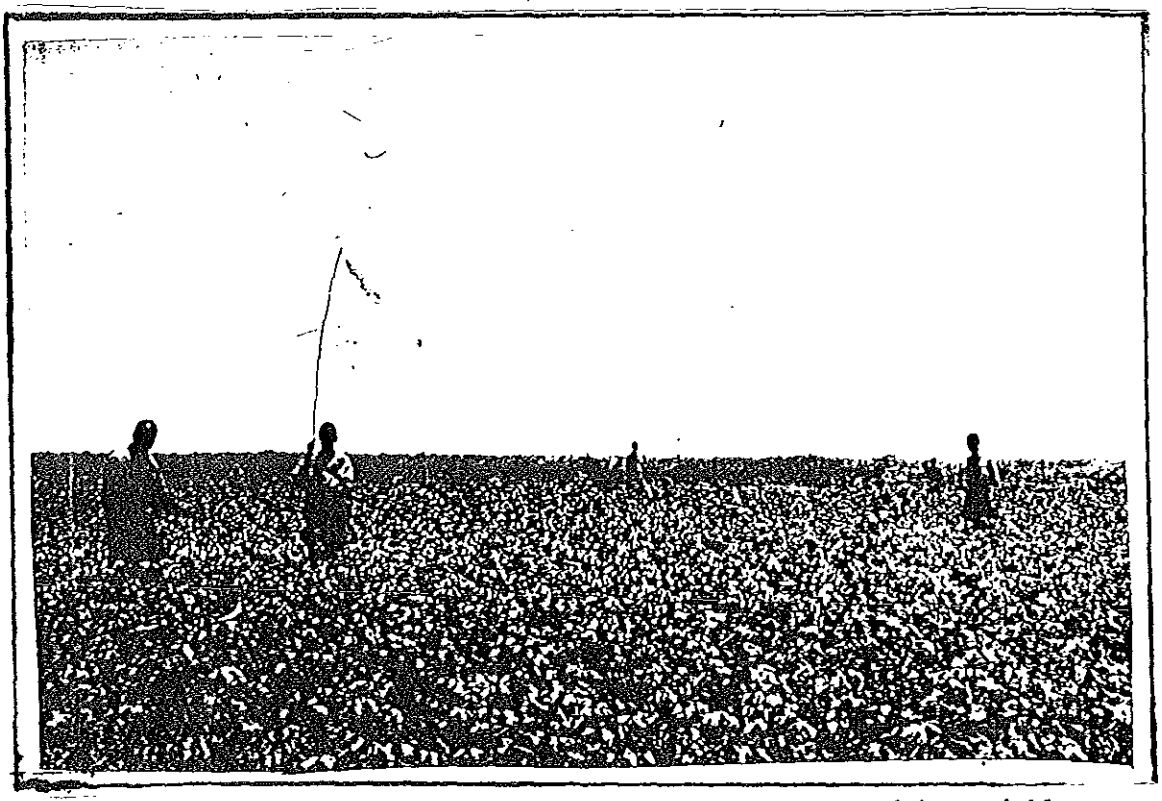
ture 1. Mixers mixing OP pesticides Guazitine and Volathion for application against cotton pests. N.Omo state farms, 1994.



Picture 2. Loaders loading an aeroplane tanker with an OP pesticide Dursban for aerial application. N.Omo state farms, 1994.



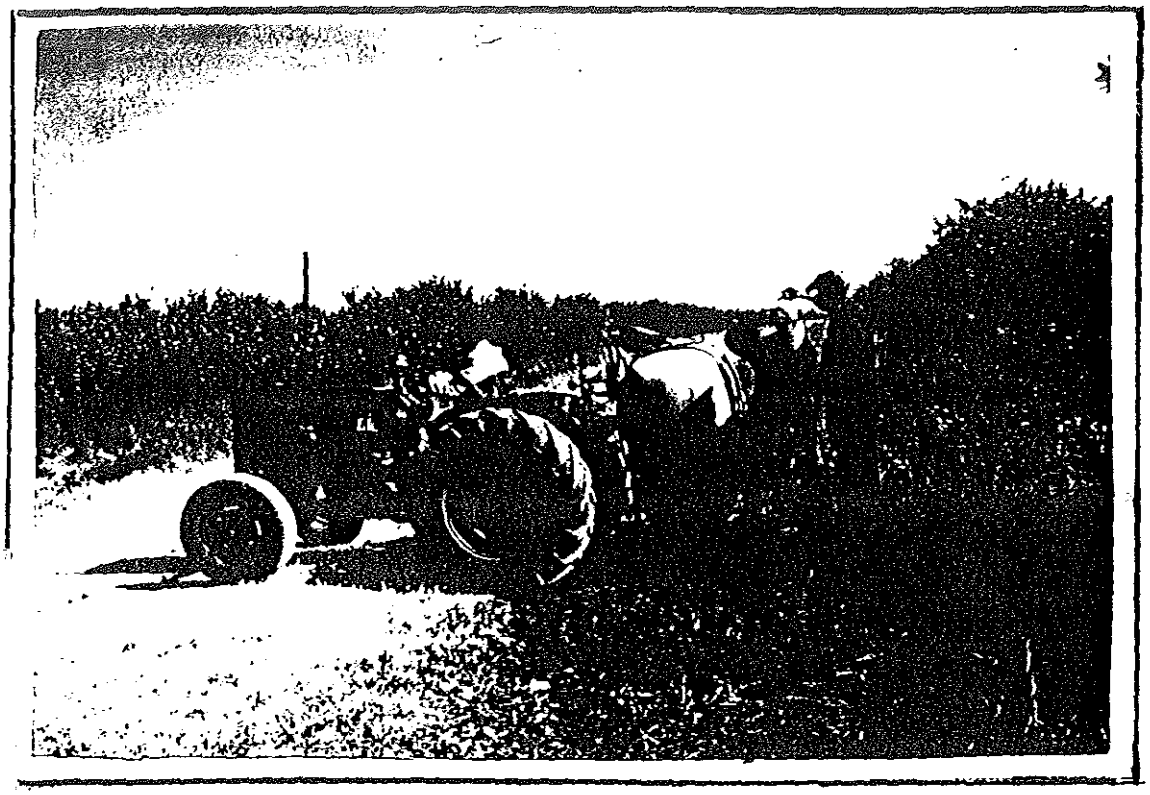
Picture 3. Aplication of pesticides on Maize and Sourghum farms by means of air crafts. Wollega state farms, 1994.



Picture 4. Flaggers guiding pilots direction during aerial pesticide applications. N.Omo state farms, 1994.

'icture 5. Organophosphates Actalic and Ultracide being applied using knapsack motorized spray machines. UAAE farms, 1994.

'icture 6. Pest assessors searching for cotton pests 24 hours after treatment with Dursban. N.Omo state farms, 1994.



Picture 7. A tractor operated machine being loaded with a pesticide to treat pests of large tree fruits. UAAE farms, 1994.

icture 8. A mechanic maintaining contaminated applicating machines at the apray field. N.Omo state farms, 1994.

Picture 9. Pest control workers use no special prequations while preparing different pesticide formulations. Wollega state farms, 1994.

Picture 10. Pest control workers commonly consume fruits around the spray fields. UAAE farms, 1994.

3.3.2 The Control Population.

A total of 161 volunteer subjects served as controls. The control subjects were selected based on the suggestions made by Maroni (1993). They were residents of Arba Minch, Addis Ababa and Nekemte, in good health and having no known environmental or occupational exposure to *OP* and *carbamate* pesticides. Most of them have occupations that require physical activities. They include employees in different factories, small scale farmers, daily labourers and office workers.

3.4 Assessement of *Organophosphate* Poisoning Symptoms and Risk Factors.

The health profile of all subjects was developed through the administration of a questionnaire. Questions which elicit information about potential causal factors of exposure that are related to pesticide application practices were also included. The questionnaire was prepared based on the WHO (1982) protocol for field surveys of exposure to pesticides and the WHO-UNDP recommendation for epidemiological study on the health effects of exposure to *OP* pesticides (Maroni et al., 1986).

The questionnaire was pretested in a pilot study and refined. It includes some basic information about their health condition, alcohol consumption, smoking and history of domestic and occupational exposure to pesticides. In addition, farm workers were questioned about the period of employment as a pesticide worker, the nature of the job, possible past poisonings or suspension from work and their work history over the last 30 days. Meteorological information such as , air temperature and relative humidity were also recorded. Since exposure to *ChE*-inhibiting pesticide produces characteristic biologic effects (Coye et al., 1986; Namba, 1971; Ciesielski et al., 1994), a fairly comprehensive range of symptoms were included in the questionnaire. Two symptoms thought not to be associated with *OP* exposure, dysgeusia and haematuria were included to assess the extent of workers recall bias. It is suggested that the scientific assessement of the impact of

pesticide use on human health does not stop with toxicology and epidemiology, but requires equal attention from the behevioral and social sciences (White, 1993). Therefore, the questionnaire also includes questions about workers knowledge, attitude and practice of pesticide use.

In all the study sites, workers were questioned only once, that is, after pesticide exposures and the method used to verify and administer the questionnaire was identical for all the subjects.

3.5. Blood Sample Collection.

Blood samples were obtained at the state farm health clinics which provide health services to the farm workers and their families. Blood samples were drawn from veins in the inner forearms using a venoject needle and a 5.0 ml sterile venoject tube containing 7.2 mg EDTA. The possibility of contamination was minimized by cleaning the skin before and after drawing the blood. Since the samples were taken after variable periods of exposure, the date and time of the last exposure and the name of the last pesticide compound were noted. The samples were thoroughly mixed to avoid coagulations. The plasma and red blood cells were then separated by centrifugation at 2000 rpm for 15 minutes as recommended by Michel (1949). Finally, samples were placed into an insulated container with frozen cool ice and transported to the laboratory, and stored at -20 °c until measurements were done.

Similar sample collection procedures were followed in all the study sites. Numerous attempts were made to take blood samples for the assessment of *ChE* levels before and after pesticide application as recommended by WHO (1982) and WHO -UNDP (Maroni et al.,1986) protocols. Several other investigators (Partanen et al., 1991; Liesivuori, 1991; Ciesielsiki et al., 1994; Rama, 1995) also strongly support this approach. However, this proved impossible in this study population. In the case of wollega state farms, it is because of the workers resistance to give blood samples more than once and the relative difficulty of

reaching the workers before spraying started. For this reason, blood sample was taken from each worker only during the peak spray season (2 to 10 September, 1994). In the UAAE farms where production is totally dependent on irrigations, pesticide application is made throughout the year. In such cases, there was no distinct time interval at which time workers have not been exposed to pesticides. Therefore, only a single post-exposure blood sample was collected from each worker (from 1 to 11 December, 1994). For similar reasons given above, in the N.Omo State farms, only a single post-exposure blood sample was collected from each worker (from September 20 to October 9, 1994). No specific time was used to collect blood samples from controls.

3.6. Laboratory Analysis.

3.6.1. *Cholinesterase* Determination.

Cholinesterase activities were measured both in the RBCs and the plasma of blood. The method used in this study is basically an electrometric method developed by Michel (1949). However, some of the modifications made to this method by Mohammed and Omer (1982) were also employed. It depends upon the measurement of the acid produced by the action of *ChE* on *ACh*. The acid production is measured in terms of the change in pH produced by enzymatic activity in a standard buffer solution over a definite period of time.

3.6.1.1. Red blood cell Determination.

Approximately 5 ml of blood was centrifuged at 2,000 r.p.m. for 15 minutes in a graduated centrifuge tube, and the plasma was separated. The cells were mixed with 2 to 3 ml of 0.9 percent NaCl and centrifuged at 2,000 r.p.m. for 15 minutes. The supernatant fluid was discarded. The cells were washed for the second time with similar volume of NaCl solution and then centrifuged for 20 minutes. The volume of the cells was recorded, and then the saline supernatant was removed to the point where the remaining volume of the cells and saline is

twice the volume of the cells alone. The cells were then mixed thoroughly with the remaining saline. After mixing, 0.4 ml of the cell suspension was hemolysed in 9.6 ml. of 0.01 % saponin solution. One millilitre of hemolysed red cell solution, representing 0.02 ml. of the cells, was added to 1 ml. of buffer solution and placed in a water bath at 25 ^{0}c. The buffer solution used for RBC determination contains 0.02 M sodium barbital; 0.004 M KH_2PO4 and 0.60 M KCl. For 1 litre of buffer the reagents were dissolved in 900 ml. of distilled water ; 28 ml of 0.1 N HCl were added while shaking the solution, and the volume was then made to 1 litre. Finally the pH of the buffer was adjusted to 8.10 at 25 ^{0}c.

After allowing 10 minutes for equilibration, the initial pH (pH1) was determined with a digital pH meter reading to the nearest 0.01 pH unit. Then 0.2 ml. of 0.11 M *ACh* solution was added with rapid mixing and the time was recorded. Simultaneously, a blank solution with no enzyme added was run to control spontaneous *ACh* hydrolysis. The enzymatic reaction was allowed to proceed for one and one half hours, and then the final pH of the solution (pH2) was determined. The time at which pH2 was measured was recorded. Finally, RBC *ChE* activity was calculated in units of change in pH expressed as ΔpH/hour.

3.6.1.2. Plasma Determination.

The plasma from each sample was diluted with water so that each millilitre of solution contains 0.02 ml. plasma. One ml. of diluted plasma was mixed with 1 ml. of the buffer solution. The buffer solution used for plasma determination contains 0.006 M sodium barbital, 0.001 M KH_2PO4 and 0.30 M NaCl. For 1 litre of buffer, the reagents were dissolved in 900 ml. of distilled water and 11.6 ml. of 0.1N HCl acid was added before diluting to volume. Then, the pH of the buffer was adjusted to 8.00 at 25 ^{0}c. After the solution is allowed to equilibrate in a water bath at 25 ^{0}c for 10 minutes, 0.2 ml. of 0.165 M *ACh* was added with mixing. Subsequent determination was carried out, and the activity was calculated, in the manner as described for RBCs.

3.6.2. Liver Function Tests.

3.6.2.1. *Alkaline Phosphatase (AP).*

Alkaline phosphatase was determined according to the recommendation of the Scandinavian committee on enzymes (SCE) (1974). The method involves the continuous measurement of paranitrophenol at 405 nm formed by the following reaction.

$$\text{P-nitrophenylphosphate} \xrightarrow{AP} \text{P-nitrophenol} + \text{Phosphate}$$

A commercially available test kit number 6391 (Bayer Diagnostics Company, Germany) containing the appropriate substrate and buffer solution was used for the assay. The kit contains three bottles each with 120 ml of Diethanolamine-magnesium chloride buffer solution and paranitrophenylphosphate substrate in the form of tablets.

The substrate solutions were prepared by dissolving each tablet in 2 ml of the buffer solution and thermostated at 37 °c in a water bath before use. The buffer solution however, was brought to room temperature before use. The components and concentrations of the substrate and reagent solution in the final reaction volume were; 1 mol/l pH 9.8 (37 °c) diethanolamine buffer; 0.5 mmol/l magnesium chloride and 10 mmol/l sodium p-nitrophenylphosphate.

From each sample, 0.02 ml of plasma was taken and mixed with 2.00 ml of the buffer solution and allowed to equiliberate in a water bath at 37 °c for 15 minutes. Then, 0.20 ml of the substrate solution was added. The final solution was mixed in a reading cuvette thermostated at 37 °c. The absorbance was read using a digital spectophotometer (model spectronic 1001). Readings were repeated four times at exactly three minutes intervals. The time intervals were controlled using a stop watch. Finally, the mean value of the absorbance changes per minute (ΔA/min) was calculated and the alkaline phosphatase activity was obtained in U/l.

3.6.2.2. *Glutamic pyruvic transaminase (GPT).*

Plasma glutamic pyruvic transaminase was determined according to the international federation for clinical chemistry (IFCC) recommendation (Bergmeyer et al., 1986). It was based on the following reaction principle.

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xleftrightarrow{GPT} \text{L-glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H+} \xleftrightarrow{LDH} \text{Lactate} + \text{NAD+}$$

A commercially available kit number 1442520 (Boehringer, Germany), was used for the assay. The kit contains nine bottles each containing 50 ml of buffer/substrate solution (bottle 1) and another bottle containing enzyme /coenzyme α-oxoglutarate reagent in the form of tablets (bottle 1a).

Initial concentrations of the reagent solution contains Tris buffer:110 mmol/l, pH 7.3; L-alanine: 550 mmol/l; Lactate dehydrogenase (LDH) = 1.3 U/l; NADH: 0.198 mmol/l; α-oxoglutarate: 16.5 mmol/l. The substance or catalytic concentration of reagent solution in the final reaction mixture was Tris buffer: 100 mmol/l, pH 7.3; L-alanine: 500 mmol/l; LDH = 1.2 U/l; NADH: 0.18 mmol/l; α-oxoglutarate: 15 mmol/l.

Reagent solution was prepared and treated according to the procedure given in the manual. Two reagent tablets from bottle 1a were dissolved in one of the 50 mls of bottle 1. After mixing, the solution was brought to a reaction temprature of 30 °c using thermostated water bath. Two ml of the reagent solution was pipetted into a 1 cm light path cuvette. Then, 0.2 ml of plasma was added. Using the blank for caliberation, initial absorbance was read at 340 nm. Reading was repeated at exactly 3 minutes interval and the mean absorbance change per minute was detrmined. The result was calculated and the activity of *GPT* in the sample was obtained in U/l.

3.7. Statistical methods

All data were edited, coded, and fed to an IBM-PC computer using the stored *dBASE IV* programe and the statistical

analysis was performed using the *SPSS* system. In general the statistical methods employed involve the following four methods.

In the first part of the analysis, *ChE* levels (both plasma and RBC), and liver function values (*AP* and *GPT*) were measured among exposed subjects and controls, and their mean values were compared. Subsequent analysis focused on the relationship of *ChE* levels among exposed subjects to specific occupational indicators such as length of employment and job categories. The third part of the analysis sought to identify the prevalence of the related symptoms and their association with *ChE* levels. The fourth and final part of the analysis considered information on the association of *ChE* activities and causal factors of potential exposure to *OP* pesticides. Analysis of variance was used to test differences between means. In the present study, $p < 0.05$, $p < 0.01$ and $p < 0.001$ were used as critical levels for statistical significance.

4.RESULTS

4.1. The study population

The age distribution of exposed subjects and controls is shown in Table 2. Of the total population surveyed, 430 (72.7%) were pesticide-exposed workers whose mean age was 32.8 yrs (range 17-55). The remaining 161 (27.2%) were the comparable controls with mean age of 33.4 yrs (range 17-51). As can be seen from table 2, both population groups have similar age distributions.

Table 2. The age distributions (the number and corresponding percentage in parentheses) of the study population (n=591).

Age group	Control (n=161)	Exposed (n=430)
< 18	7 (4.3)	4 (0.93)
18--34	84 (52.2)	262 (60.9)
35--51	70 (43.5)	161 (37.4)
> 51	--	3 (0.69)
Total	161 (27.2)	430 (72.8)

Two hundred and sixty two (60.9%) of the exposed subjects and 84 (52.2%) of the controls were between the ages of 18 and 34 yrs. This indicates that apparently young people are involved in pest control activities. Information on personal details showed that smoking, chewing tobacco and alcohol drinking were the common habits. Ninety five (22.1%) of the exposed workers and 32 (19.9%) of the controls were smokers. Alcohol consumption was high in the exposed group; 103 (23.9%) than in the controls; 14 (8.7%). None of the pesticide-exposed workers or controls reported any history of hepatic disease or other current illness. History of anaemia was recorded in 4(0.93%) and 1 (0.62%) of the exposed workers and controls respectively.

The history of their medication showed that none of the study subjects had taken anti*ChE* drugs. However, antimalaria drugs and vitamins were commonly used as most of the study sites are located in malaria endemic areas.

4.2. *Cholinesterase* Activities by different categories.

4.2.1 Overall *cholinesterase* activities of the study population.

All the *ChE* activity analyses were performed in duplicate and the averaged results were recorded. The duplicate plasma and RBC *ChE* determinations agreed within 1.8% and 2.3% in all the cases respectively. In addition, the coefficient of interindividual variation for plasma and RBC *ChE* activities among the control subjects was found to be 22.4% and 29.3% respectively.

The means, the ranges and the standard deviations of the plasma and RBC *ChE* activities determined for the exposed workers and controls are shown in Table 3. It is evident from the table that the mean plasma and RBC *ChE* activities of the exposed subjects (0.949 and 0.639 pH units respectively) were found to be significantly lower than the plasma and RBC *ChE* activities of the controls (1.049 and 0.787 pH units respectively) ($p<0.001$).

Table 3. The plasma and RBC *ChE* activities of the control and exposed subjects (values are mean ± SD; figures in parentheses indicate the corresponding range values).

ChE activity (pH units)	Control (n=161)	Exposed (n=430)
Plasma	1.049 ± 0.235	0.949 ± 0.278 *
	(0.535 -- 1.428)	(0.238 -- 1.388)
RBCs	0.787 ± 0.231	0.639 ± 0.278 *
	(0.390 -- 1.492)	(0.106 -- 1.435)

* $p < 0.001$

In addition, 55 (12.8%) and 49 (11.4%) of the exposed subjects were found to have *ChE* levels below 0.524 and 0.393 pH units (50% of the control plasma and RBC *ChE* values) respectively. Furthermore, 58 (13.5%) and 47 (10.9%) of the exposed subjects had plasma and RBC *ChE* levels below the control minimum value (0.535 and 0.390 pH units respectively).

However, no control subject was found to have the plasma or RBC *ChE* level below the lower limit of the exposed subjects (0.238 and 0.106 pH units respectively).

4.2.2 Age group.

Tables 4 and 5 depict *ChE* activities of the control and the exposed subjects with respect to the various age groups respectively. Analysis of variance showed that there were no significant difference in the mean plasma and RBC *ChE* activities of the control subjects with respect to the various age groups (Table 4).

Table 4. *Cholinesterase* activity distributions (pH units) of the control groups by age (n=161) (values are mean ± SD).

Age group	Number	Plasma *ChE*	RBC *ChE*
<18	7	1.069 ± 0.183	0.716 ± 0.216
		(0.902 -- 1.390)	(0.400 -- 0.970)
18-34	84	1.049 ± 0.236	0.784 ± 0.212
		(0.535 -- 1.421)	(0.395 -- 1.355)
35-51	70	1.048 ± 0.240	0.798 ± 0.254
		(0.538 -- 1.428)	(0.390 -- 1.492)
>51	--	--	--

No two groups are significantly different from each other at 0.05 level

A similar result was observed in exposed subjects with the exception of the those exposed groups below 18 years of age which showed a significantly lower RBC *ChE* activities compared to those in the other age groups ($p<0.05$) (Table 5).

Table 5. *Cholinesterase* activity distributions of the exposed groups by age (n=430 Values are mean ± SD)

Age group	Number	Plasma *ChE*	RBC *ChE*
<18	4	0.993 ± 0.393	0.464 ± 0.109*
		(0.416 -- 1.293)	(0.364 -- 0.583)
18-34	262	0.948 ± 0.276	0.639 ± 0.271
		(0.238 -- 1.388)	(0.165 -- 1.435)
35-51	161	0.949 ± 0.278	0.646 ± 0.285
		(0.286 -- 1.342)	(0.106 -- 1.296)
>51	3	0.974 ± 0.485	0.602 ± 0.342
		(0.414 -- 1.275)	(0.302 -- 0.975)

* = P < 0.05

4.2.3 The study sites

The *ChE* activities for both the plasma and the RBCs of the exposed subjects in comparison to that of the controls at the different study sites are presented in Table 6. In this table, differences were observed in the mean *ChE* levels among workers employed in the different state farms. The observed differences were not the same for both plasma and RBC *ChE* levels.

Table 6. *Cholinesterase* activities (pH units) of the exposed subjects and the controls with respect to the different study sites (values are mean ± SD)

ChE activity	(1) UAAE (n=180)	(2) N. Omo (n=81)	(3) Wollega (n=169)	(4) Control (n=161)
Plasma	0.964 ± 0.292 (0.413 - 1.365)	0.929 ± 0.316 (0.238 - 1.388)	0.943 ± 0.242 (0.311 -1.294)	1.049 ± 0.235 (0.535 - 1.428)
RBCs	0.589 ± 0.170 (0.166 - 1.435)	0.575 ± 0.214 (0.106 - 1.041)	0.777 ± 0.333 (0.151 -1.380)	0.787 ± 0.231 (0.390 - 1.492)

SIGNIFICANCE						
	1 vs 2	1 vs 3	2 vs 3	1 vs 4	2 vs 4	3 vs 4
Plasma	N.S	N.S	N.S	P < 0.05	P < 0.05	P < 0.05
RBCs	N.S	P < 0.01	P < 0.01	P < 0.001	P < 0.001	N.S

No significant difference in plasma *ChE* among workers of the UAAE, N.Omo and Wollega state farms were observed. Similarly, the difference in mean RBC *ChE* between workers of UAAE and N.Omo state farms were found to be statistically not significant. On the other hand, the mean RBC *ChE* levels of UAAE and N.Omo farm workers were significantly lower than the Wollega farm workers and than that of the controls. Furthermore, a significantly lower plasma *ChE* levels were noted in the UAAE, N.Omo and Wollega farm workers compared to that of the control group ($p<0.05$)(Table 6). However, no significant difference in RBC *ChE* activity was observed between the Wollega farm workers and controls.

4.2.4 Job categories.

Table 7 demonstrates comparison of the mean *ChE* activities of the exposed subjects in all the study sites in relation to the different job categories. As it can be seen from the table , there were no significant differences among the mean RBC *ChE* activities of the assessors, the tractor operators and the mechanics. However, the "spray men" were identified to have significantly lower RBC *ChE* activities than the workers in the other job categories ($p<0.001$). On the other hand, a significantly lower plasma *ChE* activities were observed in the "spraymen" and the tractor operators compared to that of the pest assessors ($p<0.01$). In both cases therefore, the "spray
men" are found to have depressed *ChE* activities than workers in the other occupational categories.

Table 7. *Cholinesterase* activities (pH units) of the exposed subjects in relation to the different job categories (values are mean ± SD)

Job cat.	Number (%)	Plasma *ChE*	RBC *ChE*
Spraymen (1)	199 (46.3%)	0.919 ± 0.297	0.548 ± 0.119
		(0.238-1.365)	(0.106-1.435)
Assessors (2)	122 (28.4%)	1.014 ± 0.256	0.720 ± 0.296
		(0.293-1.388)	(0.151-1.342)
Tr.Operators (3)	73 (16.9%)	0.903 ± 0.267	0.712 ± 0.378
		(0.313-1.307)	(0.181-1.380)
Mechanics (4)	36 (8.4%)	0.992 ± 0.221	0.727 ± 0.282
		(0.320-1.315)	(0.186-1.292)

SIGNIFICANCE

Plasma	1 and 3 vs 2 = P< 0.01
RBCs	1 vs 2,3 and 4 = P< 0.001

4.2.5 Length of employment

The relationship between the length of employment or the duration of exposure and *ChE* activities is presented in Table 8. The average duration of exposure was 8.5 yrs and they worked for 5-6 hrs per day, 6 days a week. Of the total exposed workers, 9.5% served as pest control worker for less than 1 yr, 17.9% for 1-5 yrs, 29.1% for 5-10 yrs and 43.5% for more than 10 yrs.

It is evident from Table 8 that there is no significant difference in the mean plasma *ChE* activities among the different length of employment groups. However, analysis of variance showed that the mean RBC *ChE* activities were significantly different among the groups with different length of employment (p<0.001).

Table 8. *Cholinesterase* activities (pH units) of the exposed workers with respect to the length of employment in years (values are mean ± SD).

Length of employment	Number (%)	Plasma *ChE*	RBC *ChE*
< 1 (1)	41 (9.5)	0.932 ± 0.284	0.496 ± 0.103
		(0.413--1.304)	(0.291--0.715)
1-5 (2)	77 (17.9)	0.962 ± 0.275	0.627 ± 0.272
		(0.367--1.336)	(0.199--1.435)
5-10 (3)	125 (29.1)	0.976 ± 0.274	0.684 ± 0.289
		(0.238--1.388)	(0.186--1.342)
>10 (4)	187 (43.5)	0.937 ± 0.282	0.711 ± 0.287
		(0.263--1.342)	(0.106--1.296)

SIGNIFICANCE

Plasma N.S

RBCs 1 vs 2,3,4 = P<0.001; 2 vs 4 = P<0.001.

For instance, those with duration of exposure below 1 yr had significantly lower RBC *ChE* activities than those with 5 yrs and above. In addition, workers with 1-5 yrs of exposure were shown to have significantly lower RBC *ChE* activities than those who were exposed for more than 10 yrs ($p<0.001$). Generally, workers with duration of exposure below 5 yrs had low RBC *ChE* activities. This result suggests that the workers most affected are those who have been exposed to *OP* pesticides below 5 yrs than those with longer years of exposure.

4.3. Poisoning signs and symptoms

The prevalence of different *OP* toxicity symptoms in the exposed subjects in reference to that of the controls are given in Table 9. These symptoms were more frequent in exposed subjects as compared to controls. Most of the

workers also reported that the symptoms were transient. They were relieved from such complaints whenever they had a break from a continuous pesticide exposure. As a group, the prevalence of most of the *OP* poisoning symptoms in the exposed workers were significantly different compared to those symptoms reported by the control subjects ($p<0.05$) (Table 9). The main symptoms by order of importance were, headache (19.7%), weaknesses and sweating each (16.5%), wheezing (13.9%) and blurred vision (12.8%). About 9.3% of the exposed subjects and 8.7% of the controls reported frequent episodes of abdominal cramp ($p<0.05$).

Table 9. Prevalence of *OP* toxicity symptoms in the control and the exposed subjects (the number of symptoms and the corresponding percentages are given in parentheses).

Symptoms	Controls (n=161)	Exposed workers (n=430)	P value
Abdominal cramp	14 (8.69)	40 (9.30)	p<0.05
Anorexia	**2 (1.24)**	**32 (7.44)**	**p<0.01**
Ataxia	5 (3.10)	23 (5.35)	p<0.05
Blurred vision	1 (0.62)	55 (12.79)	p<0.001
Chest tightness	2 (1.24)	19 (4.41)	p<0.05
Cough	**5 (3.10)**	**33 (7.67)**	**p<0.05**
Diarrhoea	--	16 (3.72)	--
Disgausea	--	--	--
Haematuria	--	1 (0.23)	--
Headache	5 (3.10)	85 (19.77)	p<0.001
Irritation of the eye	--	43 (10.00)	--
Lachrymation	--	22 (5.11)	--
Myosis	--	5 (1.16)	--
Nausea	4 (2.48)	24 (5.58)	p<0.05
Sweating	5 (3.10)	71 (16.51)	p<0.001
Tremor	--	5 (1.16)	--
Weakness	7 (4.35)	71 (16.51)	p<0.001
Wheezing	3 (1.86)	60 (13.95)	p<0.001

Evidences of excessive cholinergic activity, such as myosis, muscle weakness and tremor were noted only in 1.16%, 5.35% and 1.16% of the exposed workers respectively. Although most of these symptoms were also reported by the controls, they were not as frequent as in the exposed subjects. The symptoms, haematuria and disgausea, were included in the questionnaire as dummy variables to see if there is a recall bias among the study population. As it can be seen from Table 9, disgausea was reported by none of the exposed subjects and controls. However, haematuria was reported by one exposed subject and by none of the controls.

In the present study, the signs and symptoms reported by the exposed workers were compared with plasma and RBC *ChE* activities. A comparison of the RBC *ChE* activities of those of the exposed subjects who had reported frequent symptoms with those who had not reported these symptoms is presented in Table 10. From this table, a definite relationship can be observed between the different *OP* poisoning symptoms and RBC *ChE* activities. Except for one symptom (irritation of the eye), the mean RBC *ChE* activities of the exposed subjects with various symptoms were low compared to those without the reported symptoms. However, except for sweating, the differences between the mean RBC *ChE* activities in the various reported symptoms were not statistically significant.

Table 10. Comparison of RBC *ChE* activities among the exposed subjects in relation to the various *OP* toxicity symptoms (values are mean ± SD).

Symptoms (number)	RBC *ChE* activity (mean ± SD) Workers with symptoms	Workers without symptoms	P
Abdominal cramp (n=40)	0.654 ± 0.273	0.638 ± 0.276	
Anorexia (n=32)	0.615 ± 0.265	0.641 ± 0.276	
Ataxia (n=23)	0.537 ± 0.172	0.645 ± 0.279	
Blurred vision (n=55)	0.608 ± 0.271	0.644 ± 0.276	
Chest tightness (n=19)	0.563 ± 0.218	0.643 ± 0.278	
Cough (n=33)	0.579 ± 0.199	0.644 ± 0.280	
Diarrhoea (n=16)	0.547 ± 0.246	0.643 ± 0.276	
Disgausea (0)	--	--	
Haematuria (1)	0.580 ± 0.148	0.639 ± 0.276	
Headache (n=85)	0.606 ± 0.271	0.647 ± 0.276	
Irritation of the eye (n=43)	0.659 ± 0.303	0.638 ± 0.273	
Lachrymation (n=22)	0.589 ± 0.344	0.642 ± 0.272	
Myosis (n=5)	0.561 ± 0.220	0.640 ± 0.276	
Nausea (n=44)	0.574 ± 0.257	0.643 ± 0.276	
Sweating (n=71)	0.555 ± 0.202	0.656 ± 0.285	**
Tremor (n=5)	0.541 ± 0.063	0.641 ± 0.277	
Weakness (n=71)	0.575 ± 0.225	0.652 ± 0.283	
Wheezing (n=60)	0.629 ± 0.260	0.641 ± 0.278	

**P< 0.05

Table 11. Comparison of plasma *ChE* activities (mean ± SD in pH units) among the exposed workers in relation to the various *OP* toxicity symptoms.

Symptoms (numbers)	Plasma *ChE* activity Workers with symptoms	Workers without symptoms	P
Abdominal cramp (n=40)	0.912 ± 0.313	0.953 ± 0.274	
Anorexia (n=32)	0.965 ± 0.285	0.948 ± 0.278	
Ataxia (n=23)	0.809 ± 0.295	0.957 ± 0.275	
Blurred vision (n=55)	0.933 ± 0.319	0.951 ± 0.272	
Chest tightness (n=19)	0.848 ± 0.338	0.954 ± 0.275	
Cough (n=33)	1.009 ± 0.250	0.944 ± 0.280	
Diarrhoea (n=16)	0.843 ± 0.342	0.953 ± 0.275	
Disgausea (n=0)	--	--	
Haematuria (n=1)	1.106 ± 0.075	0.948 ± 0.278	
Headache (85)	0.924 ± 0.283	0.955 ± 0.277	
Irritation of the eye (n=43)	0.936 ± 0.296	0.951 ± 0.277	
Lachrymation (n=22)	0.838 ± 0.257	0.954 ± 0.278	
Myosis (n=5)	0.986 ± 0.230	0.948 ± 0.279	
Nausea (n=24)	0.883 ± 0.297	0.853 ± 0.277	
Sweating (n=71)	0.918 ± 0.298	0.955 ± 0.274	
Tremor (n=5)	0.930 ± 0.371	0.949 ± 0.277	
Weakness (n=71)	0.881 ± 0.306	0.963 ± 0.271**	
Wheezing (n=60)	0.965 ± 0.297	0.947 ± 0.275	

** $p<0.05$

As shown in Table 10, of all the complaints of possible *ChE*-related symptoms, only sweating could be substantiated in terms of significantly reduced RBC *ChE* activity ($p<0.05$). A slightly higher but statistically insignificant RBC *ChE* activity was observed in those who reported irritation of the eye compared to those who didn't report this symptom.

Similarly, the plasma *ChE* levels were compared in relation to the specific signs and symptoms of *OP* poisoning. In general, Table 11 shows that in most of the cases, the mean plasma *ChE* levels in workers who reported the symptoms were lower than the workers without the symptoms. On the other hand, an unusually slight increase in plasma *ChE* activities were observed in workers who reported myosis, anorexia, nausea, wheezing and cough compared to those workers without these symptoms (Table 11). However, the differences were not statistically significant. Weakness was the only symptom reported and was found to be accompanied by significantly lower plasma *ChE* level ($p<0.05$). Similar to the other symptoms, the mean plasma and RBC *ChE* activities of the worker who reported haematuria was not significantly different compared to those with out this symptom (Tables 10 and 11).

4.4. Liver function tests.

Comparison of the mean *AP* and *GPT* values betweeen the exposed subjects and the controls are prsented in Table 12.

Table 12. Comparison of the *AP* and *GPT* values of the exposed subjects and the controls (values are mean ± SD and the corresponding ranges are given in parentheses).

LFT (U/l)	Exposed (n=430)	Controls (n=161)	significance
AP	77.59 ± 42.99	76.15 ± 29.93	N.S
	(33.90 -- 390.90)	(32.90 -- 176.20)	
GPT	14.24 ± 9.45	14.79 ± 10.67	N.S
	(5.00 -- 90.00)	5.00 -- 110.00)	

LFT = Liver function tests

N.S = Not significant

As it can be seen from this table, there were no significant differences in *AP* levels between the exposed subjects and that of the controls. Similarly, the difference in *GPT* levels between the exposed subjects and controls was found to be statistically insignificant. The mean *AP* and *GPT* values in both the exposed subjects and the controls are found to be within the normal limits (50 to 250 U/l and 0 up to 29 U/l respectively).

4.5. Risk factors

Table 13 depicts some of the factors that are likely to affect the occurrence of pesticide poisoning in the different state farms studied.

In the UAAE, N.Omo and Wollega farms, 12.8%, 8.6% and 24.6% of the workers mentioned having suffered from previous occupational pesticide poisoning.

Most of the poisoning incidents occur within the 20 to 40-year age group. The cause of this incident could be directly or indirectly attributed to the factors indicated in Table 13.

Table 13. Risk factors likely to affect the occurrence of pesticide poisoning in the exposed groups (the number of the positive responses are given with their respective percentages in parentheses).

Risk factor	UAAE	N.OMO	WOLLEGA	TOTAL
Awareness of danger				
of poisoning	20(11.1)	13(16.0)	19(11.2)	51(12.1)
Training	8(4.4)	21(25.9)	4(2.4)	33(7.6)
Changing clothing	108(60)	28(34.6)	88(52.1)	224(52.1)
Eating at work	154(85.6)	44(54.3)	33(19.5)	231(53.7)
Washing hands				
before eating	89(49.4)	35(43.2)	100(59.2)	224(52.1)
Bathing after				
spraying	48(26.6)	24(29.6)	43.3(25.4)	15(26.7)
Using personal				
protective devices	30(16.7)	12(14.8)	36(21.3)	78(18.1)

As it can be seen from table 13, only 12.1% of the total exposed workers were aware of the danger of poisoning which is associated with pesticide handling. The workers believed that their knowledge of the health risks associated with pesticide use is adequate. They also claim that they take precautionary measures when handling pesticides. On the other hand, 87.9% of workers were unaware of the danger of poisoning and even some of them believe that they are resistant to most of the toxic pesticide formulations. There is also lack of guidance and training in pesticide use.

Only 7.6% of the total workers reported having taken training about the safe handling of pesticides (Table 13). The situation is alarming, particularly in the UAAE and the Wollega farms and shows how susceptible the workers are to the risk. Fifty two percent of the workers reported that they change their clothing after spraying. Even though this is a necessity in all the farms where *OPs* with toxicity category I and II are commonly used, 48% of the exposed subjects did not take care of this risk factor.

Likewise the habit of eating around the spray field area was common in all the study sites. Drinking and smoking during pest control activties were also widespread among the workers. Responses to the habit of eating showed that 85.6%, 54.3% and 19.5% respectively of the UAAE, N.Omo and Wollega farm workers used to eat at work. The picture is alarming specially in the UAAE farms where the workers commonly consume fruits at the spray fields (Picture 10).

Workers took no special precuations during spraying or mixing the pesticides and there is high possibility of contamination with toxic formulations (Pictures 1 and 9). Most of the pesticides used in the farms were in the "highly hazardous" or "extremely hazardous" toxicity classes (Table 1). During application of such pesticides, the use of complete protective gear is strongly recommended. Despite this, workers commonly wore their everyday clothing and spray without foot wear. Only 16.7%, 14.8% and 21.3% of the workers in the UAAE, N.Omo and Wollega farms respectively properly used personal protective apparel. The others usually wore ordinary work clothes consisting of long pants or cotton overalls, short-sleeved shirt and in some cases jackets. Gloves, chemical respirators or masks, and boots were not normally worn during the application of pesticides in the study sites.

During field observations, it was found out that workers have great interest or positive attitude towards using personal protective devices. However, they claimed that these protective devices were either not provided or the appropriate type may not be available and caused discomfort to the users. In most cases, old protective devices were not replaced on time and farm workers were mostly using tornout rubber boots, gloves or overalls.

In addition, 28.6%, 29.6% and 25.4% of the UAAE, N.Omo and Wollega farm workers bathed after spraying. This information may not be reliable with the water shortages and poor infrastractures in the farms.

The relationship between important risk factors and *ChE* activities are separately indicated in Tables 14 and 15. In general, the effect of the risk factors observed in the present study was not the same for plasma and RBC *ChE* levels. As can be seen from both tables, the habit of eating during pest control operations, was accompained by significantly lower plasma and RBC *ChE* activities($p<0.05$ and $p<0.001$ respectively).

Table 14. Comparison of the RBC *ChE* activities in pH units with respect to the different risk factors (values are mean ± SD)

Risk Factor	RBC *ChE* activity		
	Response (no)	Response (yes)	sign.
Changing clothing	0.657 ± 0.292	0.654 ± 0.259	N.S
Eating at work	0.733 ± 0.283	0.597 ± 0.232	P<0.001
Washing hands before eating	0.636 ± 0.265	0.652 ± 0.285	p<0.01
Bathing after spraying	0.638 ± 0.271	0.642 ± 0.287	N.S
Use personal protective devices	0.632 ± 0.268	0.674 ± 0.308	N.S

Sign. = significance
N.S = not significant

Similarly, exposed workers who did not wash their hands before eating had significantly lower RBC *ChE* activities than workers who have taken care of this risk factor properly (p<0.01) However, the difference observed in plasma *ChE* activity with respect to this causal factor was not statistically significant. A significant difference in plasma *ChE* activity was also observed between exposed workers with and without protective devices (p<0.001) (Table 15).

Table 15. Comparison of the plasma *ChE* activities in c- units with respect to the different risk factors (values are mean ± SD)

Risk factor	Plasma *ChE* activity		
	-Response (no)	Response (yes)	Sign.
Changing clothing	0.933 ± 0.276	0.964 ± 0.279	N.S
Eating at work	0.957 ± 0.264	0.922 ± 0.290	P<0.05
Washing hands before eating	0.950 ± 0.280	0.948 ± 0.276	N.S
Bathing after spraying	0.954 ± 0.270	0.937 ± 0.299	N.S
Using personal protective devices	0.845 ± 0.301	0.972 ± 0.268	p<0.001

Sign. = Significance

N.S = Not significant

However, there was no significant difference in mean RBC *ChE* activity in the exposed workers who used personal protective devices compared to those who didnot (Table 14). In addition, in the present study, causal factors of pesticide exposure such as changing clothing and bathing after pest control operations had no association with plasma or RBC *ChE* activity depression.

5. DISCUSSION

5.1 Cholinesterase activities by different categories

5.1.1 The study population

In the present study, the mean plasma *ChE* values found in the control group was 1.049 ± 0.235 (Table 3). This result is more or less comparable with those reported by Duncan et al (1986): 0.94 pH units; Brown et al (1978): 0.99 ± 0.17 and by Abiola et al (1988): 0.90 ± o.1. The mean RBC *ChE* activities of the control group found in the present study (0.787 ± 0.231), is in close agreement to those reported by Brown et al (1978): 0.75 ± 0.1. However, it was higher than the values reported by Maroni et al (1990): 0.595 and lower than the values reported by Abiola et al (1988): 1.80 pH units.

The common existence of wide individual differences in plasma and RBC *ChE* activities were pointed out in earlier studies by Jeyaratnam et al (1986) and Loosli (1980) respectively. In addition, Coye et al (1986) stated that there is no correlation between erythrocyte and serum enzyme activities in the absence of *ChE* inhibition. In the present study, the total variation observed between the duplicate intraindividual readings were 1.8% and 2.3% for plasma and RBC *ChEs* respectively. This was lower than the 7% variation observed in the work of Quinones et al (1976). Serat and Mengle (1973) observed a 5% variation in *ChE* activity in 61% of the sample pairs analysed under the same laboratory conditions. Yager et al (1976) suggested that laboratory variation contributes to 40% and 25% of the variation in RBCs and plasma ChE values respectively.

In general, findings of the present study showed a better match between the duplicate plasma and RBC *ChE* readings.
The coefficient of interindividual *ChE* activity variation observed among the control subjects was 22.4% and 29.3% for plasma and RBCs respectively. For the exposed subjects, the coefficient of interindividual plasma and RBC *ChE* activity was 29.8% and 43.5%. This finding is against to the opinion that in normal persons, the plasma *ChE* is more variable than the RBC *ChE* and is considered as a less reliable indicator of exposure to *ChE*-inhibiting pesticides (Knaak et al., 1978). The existence of wide interindividual variation in both plasma and RBC *ChE* activities have been suggested by many workers. For instance, Loosli (1980) and Coye et al

(1985) pointed out that individual differences in RBC *ChE* activity are common. In many studies, the coefficient of variation of plasma *ChE* is reported to range from 18.1% to 30.2%. On the other hand, Misra et al (1985) reported a 38.4% and 23.2% variation for plasma and RBC *ChE* levels respectively.

A statistically significant difference in both plasma and RBC *ChE* levels were observed between the exposed workers and the controls ($p<0.001$) (Table 3). These results suggest that the exposed workers are experiencing depressed *ChE* activities as a result of occupational exposure to *OP* pesticides. In agreement to the present findings, Quinones et al (1976); Gupta et al (1984); Misra et al (1985); Kundiev et al (1986); Desi et al (1986) and

Ames et al (1989) reported a significantly reduced *ChE* levels in workers exposed to *OPs* compared to unexposed controls. Recently, Maroni et al (1990) and Ciesielski et al (1994) also demonstrated a similar result.

Unlike these findings, Kummer and Van Sittert (1986) observed no significant *ChE* depression among Ivorian pest control workers exposed to *OP* pesticides. Abiola et al (1988) also observed no significant changes in plasma or RBC *ChE* activities among Senegalese farm workers exposed to an *OP* insecticide Sumithion.

A slight increase in *ChE* levels after *OP* exposure has even been reported by Rider et al (1958), Copplestone et al (1976) and Vasilic et al (1987). These variation in *ChE* levels following exposure to *OPs* is suggested to be due to differences in the extent of exposure of the workers to various *OPs* and to variations in the method of analysis of blood samples (Misra et al 1985).

5.1.2 Age group

In the present study, age did not appear to be an important factor to affect *ChE* activities. No statistically significant differences in plasma and RBC *ChE* activities among the different age groups of the controls was found (Table 4). Similarly, in the exposed workers, no significant difference in plasma and RBC *ChE* among the various age categories was noted (Table 5).

In conformity to this finding, Shanor et al (1951); Brown et al (1978); Jeyaratnam et al (1986) and Amr et al (1993) demonstrated the absence of any significant relationship between age and ChE activties. In the present study, however, the RBC *ChE* levels in the exposed workers with ages below 18 yrs was significantly lower compared to other age categories.

Unlike this finding, Munn et al (1983) found a significantly lower pesticide residue values in the gloves and urine of youth cohort than in the adults. They also suggested that the differential risk in children is probably more dependent on their toxicological susceptibility than on exposure-related parameters.

5.1.3 The study sites

Comparison of the study sites showed that the differences in mean RBC *ChE* activities in the UAAE and N.Omo farm workers were statistically significant compared to the workers in the Wollega farms ($p<0.05$) (Table 6). The mean RBC *ChE* activities in UAAE and N.Omo farm workers were significantly different from the controls ($p<0.05$), while the mean RBC *ChE* activities of the Wollega farm workers were not. Such differences are expected to occur and may be attributed to the differences in one or a combination of several factors: The temperature and humidity difference at which pesticide application was carried out; toxicity of the *OP* compound sprayed; differences in the extent or duration of exposure and differences in the prevalence of risk factors of pesticide exposure may also contribute to such differences (Hayes, 1975; ILO, 1979; Plestina, 1984).

In the present study, the average annual temperature was relatively higher in the UAAE and N.Omo farms (33.0 °c and 32.0 °c respectively) compared to the Wollega state farm (30 °c). Most of the toxic *OP* pesticides were sprayed in UAAE farms (Table 1). Moreover, the prevalence of causal factors of pesticide exposure may also be accounted for the observed differences in the mean *ChE* depression among the different farms. For instance, the habit of eating at the spray field was high in the UAAE and N.Omo farms where fruits are grown compared to the Wollega state farms (Table 13 & Picture 10). Furthermore, the use of protective devices during pest control operations were not common in the UAAE and N.Omo farm workers compared to the Wollega farm workers.

In general, the risk factors contributing to a greater pesticide poisoning have been taken care of by Wollega farm workers compared to the workers in the other state farms (Table 13).

5.1.4 Job categories

The results of the present study showed that the mean plasma and RBC *ChE* activities of the "spray men" was significantly lower compared to the workers in the other job categories ($p<0.001$) (Table 7). This finding may suggest that exposed workers in this job category (mixers, loaders, applicators and flag men) were highly affected from exposure to *OP* pesticides compared to the assessors, the tractor operators or the mechanics. In agreement with this finding, Spigel et al (1981) identified applicators and ware house handlers of pesticides to have a significant degree of exposure ($p<0.05$) than farmers in the other job categories.

Vasilic and his coworkers (1987) also observed high level of *OP* pesticide metabolites in urine among the mixers and the applicators compared to the mechanics and other field workers. Similarly, among Pakistan vector control workers, Baker et al (1978) noted a 46.7% depression in *ChE* activity in the spray men who were exposed to malathion formulation.

In general, the present study suggests that the 'spray men' are the most high risk groups. This may be due to the fact that these workers have frequent contact with the most concentrated technical grade of the *OP* pesticides during mixing, loading and application practices. It was also observed that workers in this job category exchanged from sections to sections as the need arises. Workers mobility may enhance the possibility of exposure to more than one pesticide formulation which may be more potent as a result of synergistic effect (Hayes, 1975; Plestina, 1984).

The tractor operators were also found to have significantly lower *ChE* activities compared to the pest assessors (Table 7). From field studies, it was observed that the tractor operators mainly operate tractor-driven 1000-L tank, fitted with a pump and spray gun used for spraying. However, tractor operators did also sometimes alternate between carrying the hose, operating the spray gun and driving the tractor. Thus, tractor operators may be exposed to about the same dose of the pesticide as the "spray men" who apply pesticides by knapsack or manual means.

5.1.5 Length of employment

In the present study, the length of employment as a pest control worker was found to affect *ChE* activity. A significant reduction in RBC but not in plasma *ChE* activity was observed in exposed workers who served for less than 1 yr ($p<0.001$). A similar result was also noted in workers who were exposed for 1 to 5 yrs compared to those with duration of exposure of 10 yrs and above (Table 8). This result was in contrast to the idea that workers exposed to *ChE* inhibitors over several years may demonstrate long-term *ChE* activity depression (Bick,1967). Roberts (1980) also mentioned the possibility that persistent exposure to pesticides may cause cumulative biological changes. In agreement with the present study, Aguilar et al (1993) observed a significant plasma and RBC *ChE* depression among agricultural workers in Bolivia who have been using pesticides for 1 to 5 yrs compared to those with longer years of exposure. This information confirms that exposed workers most affected are those who have been exposed to pesticides below 5 yrs, who have little work experience in pesticide handling. Hayes (1975) also suggested that excessive pesticide absorption occurs more likely in new employees than in those who through expeience, have learned to minimize their exposure.

Unlike the present finding, the work of Sansur et al (1990) demonstrated a significantly higher RBC *ChE* activity in *OP* exposed workers during the early period of exposure compared to those who are exposed for longer period of time.

On the other hand, in the present study, the length of employment is found to have no observable effect in the plasma *ChE* activity.

5.2 Poisoning signs and symptoms.

In the present stucy, the prevalence of poisoning symptoms were more frequent in the exposed workers compared to the controls (Table 9). The most frequently reported symptoms by order of importance were headache, sweating, weakness, wheezing, blurred vision and abdominal cramp. Likewise, the frequent occurrence of these symptoms in workers exposed to *OP* pesticides as compared to controls have also been reported earlier by many workers. For instance, Quinones et al (1976); Spigel et al (1981); Gupta

et al (1984); Borsall and Goose (1986); Wu et al (1989); Aguilar et al (1993); Lum et al (1993) and Ciesielski et al (1994), demonstrated the occurrence of one or more of these symptoms with weakness and headache being reported most frequently. In contrast to these reports, Jeyaratnam et al (1986) and Castaneda (1993) found no symptoms or clinical manifestations of poisoning among farm workers exposed to *OP* pesticides. Similarly, Sansur et al (1993) noted no symptom except fatigue among *OP* exposed workers.

What is remarkable in this study was the significantly more frequent occurrence of the commonly reported *OP* poisoning symptoms in the exposed workers than in the control group. In addition to the high prevalence of different symptoms, a positive association was observed between low RBC *ChE* activities and reported morbidity.

Despite these observations, almost all the symptoms were found to have no significant association with depressed plasma or RBC *ChE* activities (Tables 10 and 11).

Several field studies have associated *ChE* activities with signs and symptoms which are commonly known to appear due to *OP* intoxications. For instance, Amr et al (1993) reported that the symptoms of acute and chronic pesticide exposure were prominent in Egyptian pest control workers and positively associated with a significant reduction in *AChE* activity. Similarly, Kashyap (1986) and Xue (1987) observed the frequent occurrence of poisoning symptoms accompanied by significant reduction in *ChE* activities.

The work of Quinones et al (1976); Whorton and Obrisky (1983); Gupta et al (1984); Misra et al (1985) and Kummer and Van Sittert (1986) and Aguilar et al (1993) showed the absence of any significant relationship between the frequently reported symptoms and depressed whole blood, plasma or RBC *ChE* activities. On the other hand, Wu et al (1989) observed no significant relation between symptoms and plasma *ChE* activities except wheezing which was accompanied by a significant (30%) inhibition. Recently, Ciesielski et al (1994) also noted only minimal association between low *AChE* levels and morbidity. Of the frequently reported symptoms, only diarrhoea was found to be associated with significantly low *AChE* level.

Consistent with these findings, the present study also demonstrated the absence of significantly positive association between most of the reported symptoms and significant reduction in either plasma or RBC *ChE* activities. Moreover, compared to RBC *ChE* activities, the association of symptoms with plas*ma ChE* activity was not definite. This finding is in agreement with the idea that the inhibition of of RBC *ChE* is a better indicator of biological effect than plasma *ChE*, because it is analogous to the enzyme found in the nervous system tissues (Coye et al., 1986). Although not statistically significant, most of the symptoms were found to have positive association with low RBC *ChE* activities except for the complaint of irritation of the eye which was accompanied by slightly higher RBC *ChE* activities. A statistically significant difference in RBC *ChE* activity was noted between exposed workers with and without symptoms of sweating (Table 10). In addition, a statistically significant difference in plasma *ChE* activities were obseved between exposed workers with and without weakness. Therefore, in the present study, only weakness and sweating were observed and believed to have appeared as aresult of depressed plasma and RBC *ChE* levels respectively.

Generally, findings of the present study demonstrated higher prevalence of poisoning symptoms in exposed workers compared to the unexposed controls. However, the absence of strong association between most of the frequently reported symptoms and *ChE* activity reduction may occur due to several reasons.

The symptoms attributed to *OP* toxicity under chronic moderate exposure are not consistent, because their appearance depends more on the rate of fall in *ChE* activity than the absolute level of *ChE* reached (Coye et al 1986). Signs and symptoms of poisoning by organophosphorous compounds occur when morethan 50% of the plasma or RBC *ChE* activity is inhibited (Namba, 1971). It was also reported that *ChE* activity can be reduced slowly from day to day during occupational exposure to *OP* pesticides in the complete absence of signs and symptoms of intoxication (Palestina 1984). Whorton and Obrisky (1983) also observed the persistence of some significant symptoms beyond the point of recovery of measurable *ChE* activity and suggested that *RBC ChE* is an imperfect marker for events occurring in the nervous system. It was also suggested that normal workers who are not exposed to *ChE* inhibitors may unpredictably show striking fluctuation from one sample to the next.

Such fluctuations may be as great as 13 to 25% for RBC and 21 to 28% for plasma *ChE* activities (Hayes, 1982). Generally, the wide fluctuation of plasma and RBC *ChE* levels in normal persons (Misra et al., 1985; Coye et al., 1986; Jeyaratnam et al., 1986) and under-reporting and/or over-reporting of symptoms due to workers recall bias may be the causes of the absence of association between the frequent occurrence of symptoms and significant *ChE*-activity depression (Ciesielski et al., 1994). The harshness of the weather, the hardness of the nature of the work which is typical in agriculture may also be the causes of frequent reporting of symptoms.

Thus, it is not surprising to find some discrepancies between the prevalence of cholinergic symptoms and the degree of *ChE* activity reduction expected.

5.3 Liver function tests.

In the present study, no significant change in *AP* and *GPT* levels was found between the exposed workers and the controls (Table 12). This result is also in agreement with findings of Gupta et al (1979) and Misra et al (1985). In contrast to this, Bhatnagar et al (1979); Gupta et al (1984); Kashyap (1986); Kundiev et al (1986) and Ahn et al (1993) noted significantly higher *GPT* and *AP* levels in pesticide exposed subjects compared to the control groups. However, the subjects in most of these studies were exposed to a number of other types of pesticides besides *OPs*. Furthermore, in the present study, *AP* and *GPT* levels in the exposed workers were well with in the normal limits. Significant liver dysfunction in the exposed workers due to *OP* exposure seems therefore unlikely. Thus, it can be suggested that there is no marked impairment in liver function at the level of exposure assessed in the present study.

5.4 Risk factors.

In the present study, risk factors which may contribute to a greater extent of pesticide exposure were found to be prevalent among the exposed subjects (Table 13). Several epidemiological studies on workers knowledge, attitude and practice of pesticide use also demonstrated similar observations.

Surveys of the causes of pesticide exposure conducted in Bolivia by Aguilar et al (1993), in Thailand, Malaysia and Philippines by Lum et al (1993) and in the Jordan valley by Sansur et al (1993) showed that workers ignorance of the toxicity of pesticides, lack of training on the safe use of pesticides, total absence or improper use of personal protective devices and workers poor personal hygiene are highly prevalent among pesticide exposed population. They were found to be the main causes of pesticide related illnesses. Consistent with these findings, in the present study, the effects of these factors as the main causes of pesticide related poisoning may be substantiated by the strong association observed between the prevalence of these factors and reduction in the plasma or the RBC *ChE* activities (Tables 14 and 15). As can be seen from Table 14, the higher prevalence of factors like the habit of eating at work and not washing hands before eating were accompanied by a significantly lower RBC *ChE* level compared to those who have taken care of these factors ($p<0.001$ and $p<0.01$ respectively). Similarly, a significant difference in plasma *ChE* level was observed between those who properly use personal protective devices compared to those who didn't ($p<0.001$) (Table 15). In agreement with this finding, Castaneda (1993) observed a 40% reduction in plasma *ChE* level among Filipino spray men without personal protective devices; however no significant difference was observed with regard to RBC *ChE* levels. Recently, Ciesielski et al (1994) also demonstrated most significant associations between such risk factors of pesticide exposure and low RBC *ChE* levels among workers exposed to *OP* pesticides.

6. CONCLUSION

The present study has tried to assess the health impact on some Ethiopian farm workers exposed to multiple cholinesterase-inhibiting *OP* pesticides. Plasma and *RBC ChE* activity determinations, liver function tests and adminstration of questionnaire on signs and symptoms of *OP* poisoning and causal factors of pesticide exposure were carried out in each exposed worker. Similar measurements were done in control subjects. The results of each measurement were analysed in relation to the age, the different study sites and occupational histories. Based on the results obtained, the following conclusive statements could be made.

6.1 Although wide interindividual variation in plasma and RBC *ChE* levels were observed, both are found to be important in the assessment of health impacts of *OP* pesticides in the farm workers.

6.2 The *OP* exposed workers are found to have significantly depressed plasma and RBC *ChE* activities ($p<0.001$); the number of *OP* exposed workers with plasma and RBC *ChE* values below the minimum of that of the controls were 58 (13.5%) and 47 (10.9%) respectively. This indicates excessive absorption of *OP* toxicants by the exposed workers.

6.3 Of the three state farms surveyed, the UAAE and N.Omo farms are found to be with apparently greater *OP* pesticide associated problems.

6.4 The "spray men" followed by tractor operators are identified to be the most high risk groups as compared to the pest assessors and the spray machine technicians.

6.5 *Organophosphate* pesticide exposed workers with the langh of employment below 5 years are the most affected groups compared to those who were exposed for long period of time.

6.6 Normal *AP* and *GPT* levels without significant differences with that of the controls indicates the absence of marked impairment in liver function at the level of exposure assessed.

6.7 Although poisoning signs and symptoms were frequent in the exposed workers, only weakness and sweating were identified to have associations with significantly lower plasma and RBC *ChE* levels respectively.

6.8 The risk factors related to a greater pesticide exposure such as lack of knowledge about the potential hazards of pesticides, poor personal hygiene and total absence or improper use of personal protective devices during pesticide applications were highly prevalent in the farm population. Moreover, the strong positive association observed between the prevalence of these factors and the plasma or the RBC *ChE* depression confirms the potential of these factors as the causes of *OP* poisoning.

6.9 Further investigations are needed to clearly know the nature, the amount and the route of entry of the *OP* pesticides used and to determine their association with pre- and post-exposure *ChE* activities and related morbidities. Such in depth studies would possibly rule out interferences of interindividual *ChE* variations. Further study on the future economic impact is likely important.

7. RECOMMENDATIONS

In order to minimize the health hazards from the use of *OP* pesticides, the following recommendations are given.

7.1. Provision of appropriate protective device and regular supervision on their use.

7.2. Pre-placement medical examination including baseline *ChE* determination. This is primarily important to establish baseline *ChE* level which will be used as a reference value to follow individual *ChE* status after pesticide exposure. It is also useful to screen out individuals who genetically have low *ChE* levels.

7.3. Periodic medical surveillance including *ChE* determinations, hepatic and renal function tests. Follow up is essential on the frequently reported symptoms, with special emphasis on those workers who are involved in pesticide mixing, loading, flagging and application practices.

7.4. Above all, the safe use of pesticides can be promoted by creating awareness among workers who handle and apply them. This may be achieved through disseminating information about the nature and toxicity of pesticides, training about safe handling methods and promotion of workers personal hygiene using safety training. Organizing seminars and preparing posters in local languages are quite helpful.

8. REFERENCES

Abegaz B [1983]: The rate of chemicals use in development and their impact on the environment. Sinet News letter vol 6, Faculty of Science, AAU.

Abiola FA, Sere A, Sawadogo JG, Diatta F and Ly M [1988]: Cholinesterase depression among Senegalese crop protection workers exposed to organophosphorus pesticdes. *Bull Environ Contam Toxicol.* 41:483_488.

Aguilar GC, Medina H, Chichilla J, Veneros N, Aguilar M and Carranza F [1993]: Pesticide poisoning among agricultural workers in Bolivia. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 september 1990, Ottawa, Canadala.a International Development Research Center, Ottawa, Canada. pp 76_84.

Akhabuhaya J [1991]: Pestcide legislation and regestration. *African Newsletter on Occupational Health and Safety.* September, pp 42-44

Ames GR, Brown SK, Mengle DC, K ahn E, Stratton JW and Jackson RJ [1989]: Cholinesterase activity depression among California agricultural pesticde applicators. *American Journal of Medicine* 15:143_150.

Amr MM, Batanouni EM, Emara M, Mansur N, Zayat HF, Atta GA and anad A [1993]: Health profile of workers exposed to pesticides in two large_scale formulating plants in Egypt. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada pp 118_130..

Asmerom F [1995]: Occupational health and safety problems in Ethiopia. In proceedings of the workshop on occupational health and safety, 7 september 1995, Addis Ababa, Ethiopia.
Ethiopian Public Health Association, Addis Ababa, Ethiopia pp 12-15.

Bacq ZM, Capeck R, Paoletti R and Renson J [1971]: Foundamentals of Biochemical Pharmacology. New York: pergamon press, pp 1720-1791.

Baetjer AM [1983]: Water deprivation and food restriction on toxicity of paration and paraxon. *Archives of Environmental Health.* 38(3):168-171

Baker EL, Zack M, Miles JW, Alderman L, Warren MW, Dobbin RD, Miller S and Tector WR [1978]: Epidemic malathion poisoning in Pakistan malaria workers, *Lancet*, 1: 31-34

Bergmeyer HU, Horder M and Rej R [1986]: IFCC method for Alanine Aminotransferase (L-Alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). *J Clin. Chem. Clin.Biochem.* 24:481-495

Brick M [1967]: The effect on blood cholinesterase activity of chronic exposure to pesticides, *Med J Aust* 1 (21): 1066-1071

Bhatnagar VK, Saigal S, Khemani LD and Malviya AN [1979]: Suevey amongest workers in pesticide factories, *Tox. Lett.*, 10:129-132

Bonsall JL and Goose J [1986]: The safety evaluation of endosocarb, a residual insecticide for vector control. *Toxicology Letters* 33:45_56.

Brown JR, Chail FC and Stopps GJ [1978]: Human blood cholinesterase acivity in Holland Marsh, Ontario, 976. *Bull. Environm. Contam. Toxicol.* 19:617-623

Castaneda PC [1993]: Field exposure during application of rganophosphates using knapsack sprayer in the Phlippines. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada. pp 62_69.

Ciesielski S, Loomis DP, Mims SR, and Auer A [1994]: Pesticide Exposures, cholinesterase depression and symptoms among North Carolina migrant workers. *American Journal of Puplic Health* 84:447_451.

Copplestone JF, Fakhri ZI, Miles JW, Mitchell CA, Osman Y and Wolfe HR [1976]: Exposure to pesticides in agriculture: A study of spraymen using dimethoate in Sudan, *Bull World Health Org.*, 54:129-132

Ooye MJ, Lowe JA and Maddy KT [1986]: Biological monitoring of agricultural workers exposed to pesticides: I, Cholinesterase activity determinations. *Jounal of Occupatioral Medicine* 28:619_628.

Desi I, Vetro G, Csolee I, Nehez M, Zimanyi M, Ferke A, Huszta E and Nagymajtenyi L [1986]: Biological monitoring and health survillence of a group of green house pesticide sprayers. *Toxicology Letters* 33:85-89.

Duncan RC, Griffith J and 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.

Foo GS [1985]: The pesticide poisoning report: A servey of some Asian Countries. Malaysia International Organization of consumers Unions (cited in Forget, 1991-a)

Forget G [1991-a]: Pesticides and the Third World. *Jounal of Toxicology and Environmental health* 32:11_31.

Forget G [1991-b]: Pesticide poisoning as an occupational hazard. *African Newsletter on Occupational Health and Safety*. September pp 34-38.

Forget G [1993]: Balancing the need for pesticides with risk to human health. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 September 1990, Ottawa, Canada. International Development research Center,Ottawa, Canada pp 2_16.

Grob D, Garlick WL and Harvey MA [1950]: The toxic effects in man of the anticholinesterase insecticide parathion (p-ranitrophenyl Dethythionophosphate). Johns Hopkins Hosp. Bull. 87,106 (cited in Popendrof and Leffingwel 1982).

Gupta RC, Dave SK, Shah MP and Kashyap SK [1979]: A monitoring of study of workers handling pesticides in ware houses and godowns, *J Environ. Sci. Hlth.*, B14: 405-416

Gupta SK, Jani JP, Haiyed HN and Kashyap SK [1984]: Healt hazards in pesticide formulators exposed to a combination of pesticides. *Indian J Med Res* 79:666_672.

Hayes WJ [1975]: Toxicology of Pesticides. Baltimore, Williams and Wilkins pp 37-98.

Hayes W [1982]: Pesticides studied in man. Baltimore, Williams and Wilkins (cited in Coye et al., 1986).

Howard RJ, [1978]: Plasma cholinesterase activity in early pregnancy. *Archives of Environmental Health*. 33(6):277-278

ILO (International Labour Organization) [1979]: Guide to health and hygiene in agricultural work. International Labour Office, Geneva, Switzerland. *pp 94-157*

Jeyaratnam J, Lun KC and Phoon WO [1986]: Blood chlinesterase level among agricultural workers in four asian countries. *Toxicology Letters* 33:196_202.

Jeyaratnam J [1993]: Acute pesticide poisoning in Asia: the problem and its prevention. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20, september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada pp 26_30.

Kangas J and Jauhiainen A [1991]: Determination of cholinesterase activity. *African Newsletter on Occupational Health and Safety*. September 56-58

Kashyap Sk [1986]: Health servillance and biological monitoring of pesticide formulations in India. *Toxicology Letters 33:* 107-114.

Knaak JB, Maddy KT, Fredrickson AS, Peoples SA and Love R [1976]: Cholinesterase activity in blood samples collected from field workers and non field workers in California. *Toxicology and applied pharmacology* 45:775_770.

Kummer R and Vansittert NJ [1986]: Field studies on the health effects from the applications of two organophosphorus insecticide formulations by hand_held ULV to cotton. *Toxicology Letters* 33:7_24.

Kundiev YUI, Krasnyuv EP and Viter VPH [1986]: Specific feature of the changes in the health status of female workers exposed to pesticides in green houses. *Toxicology Letters* 33:85_89.

Lewalter J and Korallus U [1986]: Erythrocyte protein conjugates as a priciple of biological monitoring for pesticides. *Toxicology Letters* 33:153_165.

Liesivuori J [1991]: A new approach to evaluation of exposure to pesticides. *African Newsletter on Occupational Health and Safety.* September 54-95

Loosli R [1980]: In Tordoir WF, Van Heemstra-Lequin EAH ed, Field worker exposure during pesticide application. Elsevier, New York, NY, USA Studies in Environmental Sciences 7, pp. 93-98.

Lotti M [1986]: Biological monitoring for organophosphate_induced delayed polyneuropathy. *Toxicology Letters* 33:167_172.

Lum KY, Mamat MJ, Oseah UB, Castandea CP, Rola AC and Sirhasemi P [1990]: Pesticide sesearch for public health and safety in Malaysia, the Philippines, and Thiland. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada pp 31_48.

Maroni M, Jarvisalo J and Laferla F [1986]: The WHO_UNDP epidemiological study on the health effects of exposure to organophosphorus pesticides. *Toxicology Letters* 33:115_123.

Maroni M, Catenacci G, Galli D, Cavallo D and Ravazzani G [1990]: Biological monitoring of human exposure to Acephate, *Arch. Environ. Contam. Toxicol.,* 19: 782-788

Maroni M [1993]: Studying the health effects of pesticides on humans. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17-20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada. pp153-157

Mercier M [1993]: Evaluation and management of pesticides on a global level: the role of international program on chemical safety, In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17-20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada. pp 196-203

Michel HO [1949]: An lectrometric method for the determination of red blood cell and plasma cholinesterase activity. *J Lab Clin Med.* 34:1564_1569.

Mineau P and Keith JA [1993]: Pestcicdes and the wild life: a short guide to detecting and residue impact. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17_20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada pp 240_262.

Misra UK, Nag D, Bushan V and Ray PK [1985]: Clinical and Biochemical changes in chronically exposed organophosphate workers. *Toxicology Letters* 24:187_193.

Mohammed FK and ST Omer EV [1982]: Modification of Michel's electrometric method for rapid measurement of blood ChE activity in animals: a mini review. *Vet. Human. Toxicol.* 24:119-120

Munn S, Keefe TJ and Savage EP [1983]: A comparative study of pesticide exposure in adults and youth migrant field workers. Archives of Environmental Health, 40 (4): 215-220

Mwathi MA and Kimani VN [1993]: Agrochemicals : a potential hazard among Kenya's small-scale farmers. In proceedings of the symposium on the impact of pesticide use on health in developing countries, 17-20 september 1990, Ottawa, Canada. International Development Research Center, Ottawa, Canada pp 106-113.

Namba T [1971]: Cholinestease inhibition by organcohosphorus compounds and its clinical effects. *Bull Wld Hlth Org.* 44:289_307.

Partanen T, Kurppa K and Ngowi VF [1991]: Occupational pesticide hazards in developing countries: epidemiological considerations. *African Newsletter on Occupational Health and Safety.* September pp 46-51

Plestina R [1984]: Prevention, diagnosis and treatment of insecticide poisoning. WHO Document WHO/VBC/ 84. 889. Geneva.

Popendorf WJ and Leffingwel JT [1982]: Regulating organophosphate pesticide residues for farmworker protection. *Residue Reviews* 82: 125-192.

Quinones MA, Bogden JD, Louria DB and Nakah AE [1976]: Depressed cholinesterase acivities among farm workers in New Jersy. *The Science of the Total Environment.* 6:155-159

WHO (World Health Organization) [1982]: Field surveys of exposure to pesticides, standard protocol WHO, Geneva, Switzerland. EC/82.1.

_____ [1994] - The WHO recommended classification of pesticides by hazard and guidelines to classification 1994 - 1995. WHO, Geneva, Switzerland. WHO/PCS/94.2.

Wu YQ, Wang JD, Chen JS, Chung SC and Hwang SY [1989]: Occupational risk of decreased plasma cholinesterase among pesticide production workers in Taiwan. *American Journal of Industrial Medicine* 16:659_666.

Xue Shou-Zhen [1987]: Health effects of pesticides: A review from the prespective of developing nations. *American Journal of Industrial Medicine* 12: 169-279

Yager J, MC Lean H and Hudes M [1976]: Components of variability in blood cholinesterase assay results. *J Occup. Med.* 18: 4: 242

Zharov V, Solomon Tefera, Mengistu mekuria and Berhane Woldekidan [1981]: The status of health of Ethiopian Agricultural workers involved in the use of pesticides. *Ethiopian Medical Journal*, 19:165.