Cognitive deficits associated with long-term, low-level exposure to organophosphate pesticides: A small group study.

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LIST OF ABBREVIATIONS

OPs	Organophosphates
OPP	Organophosphate poisoning
NS	Nervous system
CNS	Central nervous system
Ach	Acetylcholine
AchE	Acetylcholinesterase
WHO	World Health Organisation
MAFF	Ministry of Agriculture Fisheries and Food, UK.
IOM	The Institute of Occupational Medicine
Dippers flu	a condition of general malaise (headaches, aching limbs, runny nose, nausea, tightness of chest, diarrhoea, increased sweating and salivation) which often follows sheep dipping
NTE	neuropathy target esterase
OPIDN	Organophosphate Induced Delayed Polyneuropathy
IS	The Intermediate Syndrome
PPC	Personal protective clothing
UCL	University College London

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DECLARATION

I wish to make the following declaration concerning this thesis:

(a) this thesis has been composed by myself.

(b) this work was carried out by myself except where help from another source has been acknowledged.

(c) this work has not been submitted in candidature for any other degree, postgraduate diploma or professional qualification.

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ABSTRACT

Organophosphate (OPs) pesticides were derived from World War II nerve gas agents and are being increasingly used around the world for a variety of agricultural, industrial and domestic purposes. Concerns have been expressed about the effects of these chemicals on human health. Chronic ill health may follow recovery from acute organophosphate poisoning, but the possibility that repeated low level exposure may cause ill health is controversial as previous research has yielded inconsistent results. As an occupational group, farmers are considered to be at risk of low level exposure only.

Method: The present study compared neuropsychological performance of 25 agricultural workers, exposed to organophosphate pesticides in the course of their work with 22 non-exposed healthy volunteers (controls) who were matched to the exposed group for age, gender, years spent in education and level of intelligence. All of the agricultural workers were involved in litigation.

Objective: To establish whether agricultural workers with a history of prolonged exposure to OPs show evidence of cognitive impairment and to determine whether the pattern of cognitive deficit relates to exposure history.

Findings: A range of cognitive and emotional problems were identified in agricultural workers. Although general intellectual ability was relatively well preserved in the exposed cohort, they obtained lower scores on tests of auditory verbal memory span, verbal learning, verbal fluency, mental flexibility, reading, visuo-spatial skill and information processing speed, than non-exposed controls. In addition, over 70% of the exposed cohort complained of clinically significant levels of anxiety and depression. They also reported a range of physical symptoms, the most prominent being fatigue, aching muscles and joints, headaches, sleep disturbance and irritability. Exposure history varied enormously amongst individuals who seemed to have similar jobs and many appeared to have a history of undiagnosed acute poisoning. This highlights the importance of taking an adequate exposure history.

Conclusions: The question of whether low level exposure to OPs causes ill health will never be resolved without agreed definitions of acute versus low level exposure, adequate assessment of exposure history and consideration of individual vulnerability factors or synergistic effects of chemical combinations that may mediate the dose-response relationship.

CHAPTER ONE: INTRODUCTION

Organophosphates – what are they ?

Organophosphates (OPs) are chemicals which have been developed for use as pesticides to control pests, weeds and plant diseases. They include insecticides, acaricides, nematicides, rodenticides, fungicides, herbicides and defoliants. Some are used as pharmacological compounds in veterinary and human medicine, particularly as antiparasitics. They are also used as industrial chemicals. Pesticide preparations may be extracted from plants or they may be synthetic and most include carrier substances in addition to active ingredients, such as solvents, that improve absorption. These inert substances frequently comprise a large part of a commercial pesticide product and it is important to note that their adverse effects may exceed those of the active ingredient (Ecobichon and Joy, 1994; WHO Report, 1990; ECETOC Report, 1988).

Classification of pesticides

Pesticides can be classified in many different ways according to the target pest; the chemical composition of the compound used (e.g. organophosphate, organochlorine, carbamate etc) or the type of health hazard involved. (e.g. organophosphate based pesticides are classified as low, moderate or high toxicity according to their lethal dose in animals). As a general rule, the World Health Organisation (WHO) considers Organophosphate based pesticides to be one of the most hazardous types of pesticide (WHO Report, 1990).

Production of pesticides: a brief history

Inorganic chemicals were used to control insects back in ancient Greece and Rome, for example Homer mentioned the fumigant value of burning sulphur. The Chinese used arsenicals as insecticides in the early sixteenth century, but it was not until the middle of the nineteenth century that systematic scientific studies into the use of chemicals for crop protection began (WHO Report,1990).

Since their first synthesis in 1854 well over 20,000 OP agents have been developed, especially during the Second World War (Jamal, 1997). Insecticidal OPs were developed in Germany and derived from WW2 nerve gas agents. They are of lower toxicity than nerve gases such as Sarin and Soman, which were manufactured for war. However, they continue to be toxic to some organisms, otherwise they would be of no value, and they are potentially neurotoxic to humans under certain conditions (Baxter, Adams, Aw et al, 2000).

Consumption of pesticides and the increasing use of OPs

During the 1940s, organochlorine insecticides were introduced to the UK market and over the next forty years many other new pesticide products were developed. The demand for organophosphate pesticides has increased over the last two decades because they are kinder on the environment and have less biopersistence (WHO Report, 1990; ECETOC Report, 1998). OP compounds seem likely to continue to be the most important type of insecticide used in developing countries and the WHO estimates that demand for them will probably increase by more than double over the next ten years. Indeed, the use of pesticides has doubled every ten years between 1945 and 1975 and almost doubled again between 1975 and 1985. If the public health problems associated with the use of pesticides are directly related to the amounts used, these figures may indicate the extent of future problems unless measures are taken to avoid or reduce adverse health effects. More than 200 OP insecticides have been introduced on to the market, representing about 45% of all insecticides (Ecobichon and Joy, 1994). Owing to their potency, they should be handled using protective measures (e.g. gloves, face-shields, waterproof clothing, breathing apparatus) to reduce exposure.

Health concerns

Concern about the effects of pesticides on human health has been growing with their increasing use throughout the world, but there is a lack of reliable epidemiological data on the impact of pesticides on human health. Cases of severe mass poisoning have

frequently been reported (Smith and Spalding, 1959; Davies, 1990; Brown and Brix, 1998) but these are rare occurrences in relation to the widespread use of pesticides. Individual case reports have also been published (Davis, Yesavage, Berger, 1978; Gershon and Shaw, 1961) but many cases of adverse effects may remain unreported as health workers may not recognize the symptoms as being due to pesticide exposure and affected individuals may not always seek medical attention (ECETOC Report, 1998; WHO Report, 1990; Ecobichon and Joy, 1994).

Only a small number of the population are likely to be exposed to high doses of pesticides, but many more may be at risk of developing chronic effects associated with long-term, low-level exposure. As pesticides are inherently toxic to living organisms, they are more likely to affect human health than other agricultural chemicals. However, the toxicity of different pesticides varies greatly (ECETOC Report, 1998; WHO Report, 1990).

Types of exposure and individuals at risk

Different groups of the population are exposed to pesticides in different ways and in different degrees. Some exposures are intentional (suicide and homicides) and some are unintentional. About 60-70% of all cases of unintentional acute pesticide poisoning are due to occupational exposure (WHO Report, 1990).

Most patients who present with pesticide poisoning are involved in the formulation of the compounds (e.g. chemical plant workers) or their application (e.g. agricultural workers, horticulturalists, pest control operators), but also include transporters, clean-up parties and government animal health inspectors.

It is estimated that approximately 3 million pesticide poisonings occur annually worldwide and pesticide poisoning is 13 times more likely in developing countries than highly industrialized ones (Rosenstock et al, 1991; Baxter et al, 2000). Factors which may influence exposure include the use of protective clothing and biological monitoring,

but individuals in developing countries and certain occupational groups (e.g. farmers) may fail to use adequate protective measures.

Organophosphate use in the United Kingdom (UK)

In the UK OPs are extensively used as pesticides in the spraying of crops and the dipping of sheep. A number of individuals have reported ill health which they attribute to OP poisoning and there is now a great deal of public controversy surrounding the use of sheep dips. However, the incidence of ill health following exposure is unknown at present. In a postal survey carried out by Malmerg, Simkin and Hawton (1999) 16% of 1000 farmers reported ill health which they attributed to OP pesticides.

In 1976 the Ministry of Agriculture, Fisheries and Food (MAFF) introduced compulsory dipping of sheep twice yearly to prevent fly strike and other insect manifestations. Between 1976 and 1983 farmers were required to dip sheep once a year and between 1984 and 1988 this was increased to twice a year. Deregulation occurred in 1992 and it is now compulsory for agricultural workers to complete a training course in the safe handling of OP pesticides.

The process of sheep dipping (please see photograph in Appendix B)

It is usual for several people to be involved in dipping sheep. Sheep are gathered and held in pens near the dipping bath. Dipping baths vary in size and construction and may be outdoors or indoors. A worker will prepare the dipping bath by filling it with water and concentrate, which he has measured out in advance. Sheep dip is purchased by the farmer as a concentrate which is a solution containing the active ingredient at high concentration (usually diazinon or proptemaphos) in an organic solvent, that needs to be diluted 1:500 or more before use. There is a very high risk of skin contamination during the diluting process. The dipping bath is replenished during the dipping process as needed. Sheep are moved along slipways and forced into the bath (the worker who does this is referred to as the 'chucker') A worker usually submerges the sheep under the water, using an implement (this person is called the 'paddler'). Occasionally a sheep

may start to drown in the dipping bath and a worker will be required to rescue it. Farm workers are regularly splashed with sheep dip as animals struggle and shake the water from their fleece. Some protective clothing is usually worn, such as wellington boots and waterproof leggings. Farm workers are often unable to rinse sheep dip from their clothes or skin until the end of the day when they return home so the risk of skin contamination is high. Those who smoke or eat during the day are at risk of ingesting OPs. After dipping, sheep are held in a pen (outside or inside) and then either released onto grassland or herded on to lorries for transport. Sheep dip fumes may evaporate as a mist from the fleece when sheep are held indoors after dipping, increasing the risk of contamination by inhalation. Sheep dip remains in fleece for up to three months after dipping so the risk of skin contamination does not cease immediately after dipping. Therefore, the handling of fleece (e.g. when shearing, rolling fleeces or examining animals prior to/at market) is hazardous (letter from the Chief Vet at Coopers Animal Health, 1988).

Prior to 1991, those using sheep dip were advised to take minimal safety precautions against exposure (e.g. rubber aprons and gloves). In 1985 the UK Health & Safety Executive published the results of a survey regarding the range and use of protective clothing in agriculture and reported that protective measures used in agriculture were untested and unsuitable. For example, rubber aprons and gloves often disintegrated after brief use because OP formulations based on organic solvents and containing phenols were liable to penetrate clothing and degrade rubber unless contamination is washed off properly (Watterson, 1997). Over time, the degree of protection recommended increased, but many farmers considered these precautions impossible to follow as they limited mobility.

OPs are used much more widely than just in sheep dips. Cattle farmers use them to eradicate warble-fly and arable farmers spray them on crops and treat stored grain with them. OP toxicity problems are therefore not restricted to sheep farmers and many other occupational groups are at risk. Crop sprayers often spend many days per year spraying

fields. This is often done in a tractor with an open cab, so that the farm worker is exposed to spray-mist as he drives up and down a field during the course of his work. As a result, many farms invested in tractors with sealed cabs during the 1990s.

Dippers flu

Farmers refer to a condition of general malaise (headaches, aching limbs, runny nose, nausea, tightness of chest, diarrhoea, increased sweating and salivation) which often follows sheep dipping, which they refer to as 'dippers flu'. It follows the time of dipping and can last for up to 48 hours. It has been proposed that dippers flu is a manifestation of acute OP toxicity, but this is considered unproven by the scientific community at present (COT Report, 1999) and is the focus of a research project to be carried out in the near future, funded by MAFF. However, there is a remarkable similarity between the symptoms of dippers flu and those associated with acute OP poisoning (please see Table 2).

Rees (1996) measured the incidence of symptoms following exposure to sheep dip in a group of 24 farmers in the UK. Absorption of organophosphate pesticide was determined by measuring plasma and erythrocyte cholinesterase and dialkylphosphate urinary metabolites of organophosphates. None of the subjects used adequate personal protective clothing as it limited mobility. Many reported symptoms of urinary frequency, diarrhoea, insomnia, headache, wheezing and tremor in the 24 hours following exposure to sheep dip. However, only two men showed evidence of mildly lowered blood cholinesterase which indicates that clinical symptoms can occur before medical tests can detect signs of acute intoxication.

Summary

Organophosphates are pesticides which are being increasingly used around the world. Concerns have been expressed about the effects of these chemicals on human health, but there is a lack of reliable data on the scale of the problem. Most individuals who present with symptoms of OP poisoning are involved in the formulation or application of

pesticide and the incidence of OP poisoning is much higher in developing countries where adequate protective measures are lacking. In the UK, farmers use OPs to destroy insects on crops and parasites on sheep and cattle. Minimal protective measures have been used in the past and many farmers report significant contamination by OPs during the course of their work, followed by episodes of dippers flu, a constellation of symptoms which has much in common with symptoms of acute poisoning. In recent years, a number of individuals have reported chronic ill health which they attribute to exposure to OP pesticides.

CHAPTER TWO: TOXICOLOGY

Quantitative aspects of toxicity

Ideally the human dose-effect and dose-response relationships should be known for OPs in order to establish safety standards, but for most pesticides this information is not known and crude measures of the dose-response relationship in animals (e.g. LD50)[1] are the only guide. Although the results of animal tests provide some indication of the potential health hazard of a chemical to human health, there is still a need to carry out human surveys because different species may have different responses to a particular chemical and also because an infrequent effect may only become apparent in a large population study as opposed to a limited number of animal experiments (Klaassen and Watkins, 1999). Furthermore, chemicals may have a multitude of health effects, other than mortality. Information about physical symptoms and/or psychological disorder is difficult to extrapolate from animal toxicity data.

Factors which influence the toxicity of OPs

There are a large number of OP compounds of differing chemical composition which have been used for a variety of agricultural, industrial and domestic purposes. Although practically all have some toxic effects on humans, the effects vary widely according to their mode of action, uptake by the body, metabolism, elimination from the body and toxicity to humans. Some pesticides have a high acute toxicity but are readily metabolized and eliminated by the body; others have lower acute toxicity but a tendency to accumulate in the body (lipid solubility is important because it enhances the ability of compounds to cross biological membranes such as the blood brain barrier); others induce persistent biological effects even at low doses. Furthermore, it is important to note that there may be genetic differences between individuals which render some people more susceptible to the toxic effects of certain chemicals than others (Baxter et al, 2000; Klaassen and Watkins, 1999; ECETOC Report, 1998; WHO Report, 1990; Ecobichon and Joy 1994; Gossel and Bricker, 1994; Costa, Richter, Li et al 2003; Costa,

[1] LD50 = the dose which would cause an effect or toxicity in 50% of a sample of animals.

Cole, Jarvik et al, 2003). For example, human serum paraoxonase (PON1) hydrolises OPs. Increased mortality has been reported in mice which had the PON1 gene removed (PON1 knock-out mice) when they were exposed to OPs at doses sublethal to wild mice (Abou-Donia, 2005). In humans there is considerable individual variation in the serum activity of PON1 and this is partly genetically determined. PON1 polymorphisms involving the amino acids at position 55 and 192 have been identified in farmers who report ill health which they attribute to exposure to sheep dip (Cherry, Mackness, Durrington et al, 2002; Mackness, Durrington, Povey et al, 2003). Adverse effects may be caused by impurities and /or other constituents of the formulated products (e.g. solvents), as opposed to the active ingredients; and there may be synergistic effects of chemical combinations (Karalliedde, Edwards and Marr, 2003). Combined exposure to other chemicals which cause oxidative stress can decrease the level required to produce neuronal damage following exposure to OPs (Abou-Donia, 2005). Animal studies have shown that when two chemicals are combined (e.g. an OP plus DEET) severe neurotoxic effects were seen in the peripheral and central nervous system and increased mortality even though safe levels of each chemical were chosen. In other words when certain chemicals are combined, even at safe levels, the end product can be more toxic than what would be predicted from the known properties of each chemical which makes up the mixture (Abou-Donia, 1996).

Table 1: Factors which can influence the toxicity of OPs

(1) The particular OP compound an individual is exposed to.
(2) Presence of impurities and /or other constituents of the formulated products alongside the active ingredient e.g. solvents.
(3) Severity, frequency and duration of exposure
(4) The route of exposure (oral, dermal, inhalation) affects the distribution and metabolism of OPs. The approximate descending order of toxicity is as follows: inhalation, intraperitoneal, subcutaneous, intramuscular, dermal and oral
(5) Use of personal protective clothing to minimize contamination.
(6) Environmental factors (such as temperature) can increase or decrease risk.
(7) Once inside the body OPs may be metabolized, stored in fat or excreted unchanged. Metabolism usually makes the pesticide more water-soluble and easier to excrete, but in some cases metabolism increases toxicity.
(8) Age, sex, physical health of the victim.
(9) Genetic predisposition can influence an individuals' reaction to OP exposure. The enzyme paraoxonase (PON1) contributes significantly to the detoxification of OPs yet 4% of the population are deficient in this enzyme (Richter & Furlong 1999; Li, Costa, Richter et al 2000; Mackness et al 2003).
(10) Interaction with other medication or chemicals.

Acute poisoning – The cholinergic crisis

Organophosphates can be absorbed rapidly through the skin, lungs, gastrointestinal tract and conjunctiva. Once absorbed their principle action is to inhibit acetylcholinesterase, which can result in changes in peripheral, autonomic and central nervous system function (cholinergic crisis). The immediate effects of OP poisoning which occur within hours of exposure have been well documented (ECETOC Report, 1998; WHO Report, 1990; Baxter et al, 2000; COT Report, 1999, Royal Colleges' Report, 1998) and are described below.

Within the human nervous system (NS) impulses are transmitted across the junction from nerve to muscle and from nerve to nerve by means of a chemical neurotransmitter. One such important substance which transmits messages across many junctions in the

NS is acetylcholine (ACh). There are also nerve networks in the brain which rely on ACh as a transmitter. Nerves which rely on ACh for transmission are called cholinergic nerves (Kolb & Wishaw2001).

When the brain sends an impulse down a cholinergic nerve, the transmitter ACh is released at the end of the nerve. It attaches itself to a receptor on the adjacent neuronal cell or muscle to which the impulse is to be transmitted and transmission occurs. This takes about a thousandth of a second. If the process were to take longer, there would be over-stimulation of the receiving nerve or muscle. So the body provides an enzyme, called acetylcholinesterase (AChE), which breaks down the ACh and stops it from working as a transmitter. OPs act to inhibit the enzyme AChE and bind irreversibly to it. The result of exposure to such compounds is the over-stimulation of nerves and muscles followed by paralysis of transmission once supplies of ACh have been depleted (Ecobichon and Joy, 1994; Royal Colleges' Report, 1998; Karalliedde, Feldman, Henry and Marrs 2001).

Clinical symptoms and recovery

In cases of mild poisoning the first symptoms are usually fatigue, headache, weakness, salivation, dizziness, sweating, rhinorrhea, tightness in chest (due to bronchoconstriction), excessive bronchial secretions, wheezing and coughing. The symptoms are not unlike influenza and are frequently not recognised as being caused by exposure to OPs.

Moderate poisoning may result in excessive salivation, abdominal cramps, nausea, vomiting, muscular tremors, bradycardia, lowered heart rate and blood pressure.

Severe poisoning may result in constriction of the pupils, respiratory difficulty, cough, pulmonary oedema, cyanosis, diarrhoea, urinary and faecal incontinence, raised heart rate and blood pressure, convulsions, coma, cardiac arrest and possibly death

(ECETOC Report, 1998; WHO Report, 1990; Baxter et al, 2000; COT Report, 1999, Royal Colleges' Report, 1998).

Table 2: Symptoms associated with different degrees of OP poisoning

Severity	Signs and symptoms
Mild	Fatigue, headache, weakness, salivation, dizziness, sweating, rhinorrhea, tightness in chest (due to bronchoconstriction), excessive bronchial secretions, wheezing and coughing, numbness of extremities.
Moderate	Excessive salivation, abdominal cramps, nausea, vomiting, muscular tremors, weakness, difficulty talking, fasciculations, bradycardia, lowered heart rate and blood pressure
Dippers flu	*headaches, aching limbs, runny nose, nausea, tightness of chest, diarrhoea, increased sweating and salivation*
Severe	Loss of pupillary light reflex, unconsciousness, respiratory difficulty, incontinence, raised heart rate and blood pressure, convulsions, coma, cardiac arrest and possibly death.

Recovery from mild poisoning may occur rapidly in a matter of 24-48 hours; and this is why it has been assumed that OP poisoning does not cause long-term damage. It is widely believed that if an individual survives the initial life threatening crisis they will make a complete recovery. However, patients have been presenting to clinicians with symptoms which have persisted long after resolution of the cholinergic crisis. The subtle delayed effects of OP poisoning on both the central and peripheral nervous system are not well known or understood and may be unrelated to the cholinergic effects. For example, animals given toxic doses of OPs have neuropathological lesions characterized by axonal degeneration in various regions including cerebral cortex (motor and somatosensory), basal ganglia, thalamus, hippocampus and cerebellum. Time course studies have found that lesions extend into brain areas that were not initially affected, for up to 1 year following exposure, as a result of delayed apopotic neuronal cell death (i.e. programmed cell death involving free radical generation and oxidative stress) (Abou Donia, 2005). One well characterized outcome of exposure to OPs which is unrelated to AChE inhibition, is organophosphate induced delayed polyneuropathy which is related to prolonged inhibition of a particular esterase (see below).

Long-term damage

Recent research suggests there may be long-term changes in nervous system function following cessation of the cholinergic effect. OPs are capable of producing several delayed physical and neurological syndromes. The mechanism by which OPs cause these delayed effects is unclear and the focus of current research (Jamal, 1997), but is not thought to be related to the cholinergic effects of OPs.

(1) The Intermediate Syndrome (IS) was identified by Senanayake and Karalliede in 1987. It follows successful treatment of an acute cholinergic crisis. The syndrome is reported fairly frequently in developing countries and consists of proximal flaccid limb paralysis typically starting 1-4 days after poisoning. The effects last from 5-18 days. One explanation of IS is persistent blockade of the neuromuscular junction but it seems that muscle necrosis may be implicated.

(2) *Organophosphate induced delayed polyneuropathy (OPIDN)* is a delayed sensory and motor polyneuropathy affecting predominantly the lower limbs, but in severe cases the upper limbs as well. Onset is 2-4 weeks after exposure. Degeneration of the distal ends of longer axons is followed by myelin breakdown, Schwann cell proliferation and macrophage accumulation. OPIDN does not appear to be related to the anticholinesterase action of OPs, but rather the phosphorylation, ageing and subsequent inhibition of an enzyme in neurons called neuropathy target esterase (NTE). Recovery is slow and often incomplete, particularly in the central nervous system (CNS) where changes are often present in the medulla oblongata of the brain, spinal chord and CNS which seem irreversible (Jamal, 1997; Royal College's Report, 1998). Not all OPs cause OPIDN and regulatory bodies in the Western World no longer allow OP pesticides causing polyneuropathy to be marketed. However, sheep dip products contained OPs that can cause OPIDN (chlorpyrifos and coumaphos) until 1989 and 1991 respectively.

(3) *A neurobehavioural syndrome* involving subtle cognitive impairment, greater psychiatric morbidity, chronic fatigue and minor sensory changes. How OPs might cause

such effects is unknown, but several mechanisms have been proposed; such as changes in receptor sensitivity, non-cholinergic effects (e.g. on dopaminergic or adrenergic sites), inhibition of other enzymes and proteins (Jamal, 1997) and hypoxic brain damage. For example (1) Baze (1993) reviewed available published and unpublished technical reports on Soman (a nerve gas) induced morphological changes in primates. Lesions, characterised by neuronal degeneration and necrosis were seen in frontal cortex, entorhinal cortex, amygdaloid complex, caudate nucleus, thalamus, and hippocampus. They resembled the location and appearance of lesions following hypoxic brain damage. These areas of the brain are known to be involved in cognition (particularly memory and executive function) arousal and mood regulation. Lesions in non-neural tissue were found in heart and skeletal muscle. (2) Prendergast, Terry and Buccafusco (1997 and 1998) examined the effects of low-level exposure to organophosphates on memory functioning in rats and found that chronic exposure to OPs, insufficient to elicit symptoms of cholinesterase toxicity, impaired new learning in rats but not prior learning/knowledge. This impairment persisted even after withdrawl from OP exposure. AChE activity in the frontal cortex and hippocampus (areas which are known to be important for learning and memory) was suppressed and hippocampal AChE activity in the hippocampus recovered at a much slower rate than other brain regions. (3) Apopotic neuronal cell death has been reported in animals exposed to OPs, in various brain regions including cerebellum, cerebral cortex (motor and somatosensory), basal ganglia, thalamus and hippocampus and (Abou-Donia, 2005). The latter are known to be involved in cognition, particularly memory.

Summary

To summarise, four syndromes are recognised relating to the toxicity of OPs (COT Report, 1999, Royal Colleges' Report, 1998): (1) the acute cholinergic syndrome (2) the intermediate syndrome (IS) (3) OPIDN and (4) neurobehavioural effects. These four syndromes can occur independently of one another and may have different underlying mechanisms (Abou-Donia, 2005). IS and OPIDN usually follow an episode of acute poisoning. Neurobehavioural deficits have been associated with low-level exposure (in

the absence of a history of previous acute poisoning), in addition to a history of acute exposure, though the former is controversial as studies have yielded inconsistent results. A large number of factors have been identified which can influence the toxicity of OPs including individual susceptibility/vulnerability and synergistic effects of chemical combinations; yet level of exposure is frequently assumed to be the only biologically relevant/critical variable. Studies need to take account of the considerable number of variables which can influence toxicity in order to reach an understanding of the relationship between exposure and health. Pesticides have been implicated in various disorders and diseases including cancer, peripheral neuropathies (Jamal, 1997) neurobehavioural problems (Brown and Brix, 1998; Davies, Ahmed and Freer, 2000; Kamel and Hoppin, 2004), impaired auto-immune system function (Abou Donia, 2000), impaired autonomic function (Julu, 2000) and allergic sensitization reactions. There is a lack of reliable epidemiological data regarding the number of people who report ill health following low-level exposure and the nature of their problems.

CHAPTER THREE: LITERATURE REVIEW

This chapter reviews studies relating to the neurobehavioural (particularly the psychological) effects of exposure to OPs on human health. Relevant studies from the 1960s onwards were identified from a number of sources including bibliographic databases (e.g. Medline, PsychInfo), internet search engines (e.g. Google scholar), government working party reports and examination of references cited at the end of articles.

This review will discriminate between (a) neurobehavioural effects which may follow one or more episodes of acute intoxication and (b) the neurobehavioural effects following long-term, low-level exposure which does not result in discrete episodes of intoxication. These are fundamentally different situations which are frequently confused in the literature, for example, studies purporting to be examining the chronic effects of low-level exposure have often included individuals with a history of acute intoxication. The following methodological considerations need to be borne in mind when reviewing the results of previous research and studies were evaluated according to these key criteria (although not all studies provide data on all of these criteria):

(1) There is no clear cut definition of 'acute' and 'chronic' exposure. The term 'acute' is used to refer to high level exposure, sufficient to cause physical symptoms of intoxication (see Chapter Two) and most researchers classify study participants as having a past history of acute poisoning if they sought medical help for their symptoms. However, factors other than severity of illness, determine whether individuals seek medical help. For example, awareness and attribution of symptoms, health beliefs, access to medical care, belief in the benefit of medical intervention, personality and coping style (Pitts and Phillips, 1991). Indeed, a number of studies have identified agricultural workers who report symptoms of acute poisoning for which they did not seek medical help (Ohayo-Mitoko et al, 2000; Stallones and Beseler, 2002a; Salvi et al, 2003; Fletcher, MacLehose, Hurley et al, 2005). If the

definition of acute exposure revolves around whether affected individuals seek medical help (rather than asking whether ill health was experienced immediately after exposure to OPs) then mild to moderate pesticide poisoning may go undiagnosed (Jamal, 1997; Manani, Ariena, Van Bruggen et al, 2005) and there is a risk of including individuals with a history of acute poisoning in studies exploring the effects of low-level exposure. There are many uses of the term 'chronic' in the literature including persistent ill health following acute exposure, the effect of long term exposure on health (irrespective of whether it results in episodes of acute toxicity) and the effect on health of repeated low-level exposure which is not sufficient to cause symptoms of acute poisoning. With regard to the latter, studies seldom provide sufficient exposure history to convince the reader that the study participants do not have a history of acute poisoning. Widespread confusion about the definition of these terms makes it difficult to interpret the literature on the health effects of long term, low-level exposure to OPs.

(2) In many studies information concerning exposure is inadequate, both in terms of the level, frequency, duration of exposure and the compounds involved. Not surprisingly it is extremely difficult to obtain retrospectively, reliable exposure data. It is impossible to quantify the actual dose that an individual has received and the presenting signs and symptoms are likely to be the only data available on the magnitude of exposure. The level of protective clothing used may be an important mitigating factor, yet this is seldom reported on.

(3) The results from different studies may not be comparable because different occupational groups have been assessed with different exposure histories and usage of protective clothing (PPC), e.g. ground pesticide applicators, greenhouse workers, fruit-tree sprayers, farmers involved in sheep dipping. Some groups will have been exposed dermally, others by inhalation. Some have minimal exposure due to greater use of protective clothing. In California, OP pesticides are considered to be very dangerous and US law requires pesticide applicators to be monitored and a large

number of protective measures to be in place [2]. Thus, individuals who work in California are afforded a higher level of protection than those from other countries, and this should be kept in mind when reviewing studies from the USA.

(4) Different time frames of analysis have been used with some studies examining test performance before and after a single season of pesticide use whilst others have been looking for changes over a lifetime.

(5) Studies tend to focus on people who are still in employment, and may underestimate risk by excluding those with more serious health problems who have retired on ill health grounds. Sometimes the individuals in the cohorts come from different cultural backgrounds and speak different languages, which means they may not be comparable and raises questions about the validity of neuropsychological test results, given that many psychometric tests are standardised on white, English speaking populations.

(6) Variable test batteries have been used with differing levels of sensitivity. Diagnostic instruments (e.g. DSM-IV) and/or clinically sensitive, valid and reliable psychometric tests are often lacking. The limitations of various measures are seldom discussed, yet the tools of medicine are frequently less sensitive than is desirable (Baxter et al, 2000).

(7) The possibility that a sub-group of people may be particularly vulnerable to the effects of OPs has not been considered, yet inter-individual variability in susceptibility to the adverse effects of chemicals may be important at low-level exposure.

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[2] All mixing and loading of OP concentrate must be carried out in closed systems, wash and change facilities must be provided and medical supervision of blood cholinesterase activity is undertaken

Studies in individuals with a history of acute OP poisoning

Please see Table 3 for a summary of this section.

Neuropsychological

Metcalf and Holmes (1969) report on a multidisciplinary study, carried out by the University of Colorado during the 1950s and 1960s, of industrial and agricultural workers acutely exposed to a variety of pesticides. In 1952 psychometric testing carried out 72 hours after exposure revealed erratic and slowed functioning. In 1965 psychometric testing revealed that exposed subjects (n=70) obtained lower scores on tests of memory and attention, than controls. Information concerning the precise relationship with work history, severity, type and duration of exposure was not provided. Psychiatric interviews in 1952 comparing 56 exposed subjects with 22 controls found that the exposed group complained of forgetfulness, difficulty in thinking, visual difficulty, persistent muscular aches and pains and 45% of the exposed group also complained of drowsiness, fatigue and loss of interest in work, compared to only 5% of the controls.

Savage, Keefe, Mounce et al (1988) identified 100 individuals from poisoning rosters in Texas and Colorado who had a history of acute poisoning which had occurred on average 9 years earlier, having excluded anyone with a significant past medical history from further study. Each individual was matched to a control subject for age, sex, level of education, socioeconomic status and race/ethnic background. Each participant underwent a physical, neurological, neuropsychological and EEG examination and the examiners were blind to whether subjects had a history of exposure at the time of examination. Exposed subjects obtained lower scores on tests of psychomotor speed, auditory verbal memory span, mental flexibility (Wisconsin Card Sort), vocabulary, single word reading and spelling. This pattern of impairment suggests cerebral dysfunction lateralised to the left hemisphere. Twice as many exposed subjects as controls had Halstead Reitan Battery summary scores in the range characteristic of individuals with cerebral dysfunction. Exposed subjects also reported higher rates of

anxiety. No significant differences were found between controls and exposed subjects on any of the medical tests. A total of 10 different OPs were listed as the primary cause of poisoning, but no other information about severity, duration, lifetime history of exposure is given. It is possible that differences between controls and poisoned subjects may have resulted from incomplete matching on verbal intelligence. Nevertheless, the findings suggest that a history of acute poisoning is associated with cognitive impairment.

Kurlychek and Morrow (1989) examined 7 greenhouse coworkers with a history of exposure to pesticides who reported ill health, which they attributed to OP poisoning. They were compared with 7 controls matched for age and years in education. Significant differences were seen between exposed and non-exposed workers on tests of attention (digit span), mental flexibility (Trails B), psychomotor speed (digit symbol, TrailsA), visual and verbal learning. No differences were found on tests of perceptual-motor coordination.

Rosenstock, Keifer, Daniell et al (1991) examined 36 farm workers from Nicaragua who had been admitted to hospital about 2 years earlier with acute OP poisoning (having excluded anyone with a significant past medical history). They were compared with age matched controls. Unlike Savage et al they found no significant differences in vocabulary scores between the groups. However, exposed subjects obtained lower scores on tests of digit span, digit symbol and visual but not verbal memory. These results suggest the findings of Savage et al can not be attributed solely to the failure to match control and exposed subjects adequately. Yet again, the type of OPs used and severity of poisoning are not reported.

Reidy, Bowler, Rauch et al (1992) examined 21 migrant hispanic farm workers acutely exposed to OPs on two occasions and compared them to 11 matched controls. Results revealed that 2 years after exposure, the exposed group were more impaired than controls on tests of psychomotor speed, dexterity and visual memory. They also reported more symptoms of anxiety and depression . Detailed information about lifetime

exposure history is not provided. The farm workers were involved in litigation, but re-assessment following settlement of their claims found no improvement in symptomatology or mood state (cognitive function was not formally assessed at this stage).

Misra, Prasad and Pandey (1994) examined 32 Indian pesticide applicators (mean duration of exposure was 10.5 years) and compared them to 25 controls (matched for age, education, social class). The workers were examined after spraying and underwent a medical examination, psychometric testing and measurement of event related potentials. Blood cholinesterase levels were assessed (serum AChE) to measure recent exposure. Workers had lower AChE levels than controls and reported a range of symptoms immediately after spraying including headache, giddiness, eye irritation, paresthesia, insomnia, depressed mood. They preformed more poorly on a visuo-spatial construction test, visual and verbal memory tests. Perseveration was the most common abnormality detected during testing. P3 latencies were prolonged in comparison to controls, but within normal limits. There was no relationship between cognitive function and serum AChE levels and the authors conclude that changes in cognitive function are related to long term exposure to OPs. However, limited information about previous exposure history is provided in this study.

Steenland, Jenkins, Ames et al (1994) compared 128 men in California poisoned by OPs between 1982 and 1990 with 90 controls. Tests included a neurological examination, nerve conduction tests, vibrotactile sensitivity tests and 10 psychometric tests. The poisoned group performed significantly worse than controls on only one test, visual sustained attention.Those who had been more severely poisoned and required hospital treatment also performed poorly on a test of psychomotor speed (digit symbol). The psychometric test battery used in this study was very limited and may have lacked sensitivity.

Psychiatric

Psychiatric abnormalities have been reported following acute OP poisoning and include anxiety, depression, irritability, emotional lability, fatigue, insomnia, excessive dreaming and cognitive impairment.

Gershon and Shaw (1961) looked at the incidence of psychiatric problems in sixteen individuals with a history of OP exposure (duration of exposure ranged from one and a half to ten years). Seven individuals were diagnosed with depression and 5 with psychosis. One was in a fugue state and the remaining individuals complained of cognitive impairment (as did everyone to some degree). Eleven had a history of acute poisoning and safety precautions (i.e. PPC) were frequently neglected. Follow-up showed the effects persisted for 6 months after exposure had ceased, but almost returned to normal within 12 months. Past medical history was taken into account before Gershon and Shaw concluded that these psychiatric symptoms were related to OP exposure. However, they did admit that this association could have occurred by chance.

Amr, Halim and Moussa (1997) examined 208 Egyptian pesticide formulators, 172 pesticide applicators and compared them to 233 controls (matched for age, social class and education). Formulators and Applicators had been exposed to a range of pesticides (including OPs, organochlorines, carbamates and synthetic pyrethroids) for at least 2 years. All study participants were assessed by a psychiatrist with reference to DSM-III-R criteria and completed the GHQ. Psychiatric disorders were significantly higher among pesticide formulators and applicators than controls and in those with a longer duration of exposure (e.g. more than 20 years). Furthermore, the incidence of reactive depression was nearly equal in all groups, but the incidence of neurotic or dysthymic disorder was higher in exposed subjects than in controls and higher than that seen in the general population of Egypt. The authors conclude that the increase in psychiatric morbidity relates to the cholinergic effects of pesticides. A major weakness of this study is the failure to provide any information about exposure history, other than to describe the exposed subjects as having heavy and continuous exposure. It is impossible to determine

whether they have a history of acute poisoning. Furthermore, the authors missed an opportunity to compare applicators with formulators directly. Working practices, use of protective clothing and routes of exposure may differ in these groups.

Stallones and Beseler (2002a; 2002b) carried out a cross sectional survey of 761 farmers and their spouses in Colorado and found that this population of farm residents had lower rates of depression than the general population. However, those with a history of exposure to pesticides in high enough concentrations to cause self reported symptoms of poisoning were more likely to be depressed. They were also more likely to report neurological symptoms including poor memory, concentration and language ability, irritability, heart palpitations, headaches, hypersomnia and difficulty grasping things. The odds of an acute pesticide related illness were higher in those exposed to OPs and in crop sprayers as opposed to those who apply pesticides to livestock.

Salvi, Lara, Ghisolfi et al (2003) examined 37 Brazilian tobacco growers exposed to OPs for 3 months and re-assessed them 3 months later when they were no longer exposed. All participants underwent a clinical examination and completed the mini mental state test (MMSE). Plasma AChE levels were within normal levels and were not different between on and off periods of exposure. 52% of workers had a history of mild acute poisoning (though not in the past year) but only one person sought medical help. All subjects obtained MMSE scores within the normal range. 12 subjects had clinically significant extrapyramidal symptoms and 48% had a psychiatric disorder during exposure (though this figure decreased when exposure ceased). The authors conclude that exposure to OPs may cause significant neurological and psychiatric symptoms and that tests other than AChE activity are needed to monitor the effects of long term low-level exposure. Limitations of this study include the lack of any control group; the only test of cognitive ability was the MMSE which is not sensitive to mild or focal cognitive deficits; and limited information about exposure history was provided.

Summary

The majority of studies find some evidence of cognitive impairment following previous episodes of acute poisoning. Attentional deficits, psychomotor slowing and greater vulnerability to psychiatric disorder (particularly anxiety and depression) are most common. Less frequently, additional problems such as impaired working and visual memory, language, and executive functioning (associated with frontal lobe dysfunction) have been reported.

In 1998 a working party was commissioned by the Department of Health in the UK to review the scientific evidence concerning chronic ill health following exposure to OPs. The working party reported to a committee on toxicity of chemicals in food, consumer products and the environment (COT). Following their review of the literature, the COT report concludes that studies provide "reasonable, although not conclusive, evidence that OP poisoning of sufficient severity to require hospital admission can lead to persistent cognitive impairment. This effect is most evident in neuropsychological tests involving sustained attention and speeded flexible cognitive processing, for example the digit symbol substitution test".

It is now generally accepted that long-term neurobehavioural effects may follow recovery from one or more episodes of acute poisoning, but these problems may be sufficiently subtle to be overlooked during routine clinical examination (Brown and Brix, 1998).

Table 3: Studies of individuals with a past history of acute OP poisoning.

Reference	Type of study	Subjects	Tests/functions	Exposure	Result
Metcalf & Holmes 1969	Follow-up study of industrial and agricultural workers acutely exposed to Ops	56 exposed 22 controls	WAIS Benton visual retention Story recall	Relationship to work history and exposure not reported.	Erratic & slowed functioning seen 72 hours after exposure. Follow-up 13 years later found exposed subjects had memory and attentional deficits.
Savage et al 1988	Group comparison Investigators blind to exposure status	100 individuals with physicians diagnosis of OP poisoning in Texas, USA. 100 matched controls (age, sex, race SES, edu)	Halsteid Reitan Battery. WAIS Language Executive Function Story recall Motor coordination Perception	Acute poisoning on average 9 years earlier with a range of OPs. Subjects were from a range of occupations; farm workers, pilots, manufacturers.	Exposed subjects had lower scores on tests of psychomotor speed, memory span, mental flexibility, vocabulary, reading & spelling. Also higher rates of anxiety.
Kurlychek & Morrow 1989	Group comparison	7 Greenhouse workers referred for medical evaluation 7 age matched controls	Block design Digit symbol Digit span Information Similarities WMS-R verbal and visual learning subtests Trails A&B Manual dexterity	Subjects exposed to a variety of chemicals. Employer was in violation of safety regulations.	Exposed subjects had lower scores on tests of digit span, digit symbol, Trails A, mental flexibility (Trails B), visual & verbal learning.
Rosenstock et al 1991	Group comparison	36 Nicaraguan farm workers admitted to hospital with acute OPP 36 age matched controls	RT Digit span Digit symbol Manual dexterity Benton VR test Rey AVLT Finger tapping Trails A Vocabulary Block design	Acute poisoning on average 2 years earlier	Exposed subjects had lower scores on tests of sequencing and problem solving, visuomotor speed, verbal memory span, visual memory tests, RT and dexterity. No difference in vocabulary scores noted.

Reidy et al 1992	Follow-up study over 2 years	21 migrant hispanic farm workers All litigants 11 matched controls (age, sex, educ, SES, ethnicity)	*CNS/B-I Battery* Digit span Digit symbol Vocabulary Arithmetic Block Design Trails A&B Benton VR test WMS	Acutely exposed on 2 occasions to a combination of mevinphos, methomyl & maneb.	2 years later exposed subjects had lower scores on tests of psychomotor speed, dexterity and visual memory. Also higher rates of anxiety and depression.
Misra et al 1994	Group comparison after spraying	32 Indian pesticide applicators 25 controls	Medical Exam ER Potentials Benton VRT WMS Alexander Passalay T Finger Dexterity	Evidence of cholinesterase inhibition. Clinical symptoms reported after spraying. Average of 10 years exposure to OPs.	Exposed subjects obtained lower scores on visual and verbal memory tests, not related to cholinesterase inhibition. P3 latencies were prolonged.
Steenland et al 1994	Group comparison Investigators blind to exposure status	128 cases of accidental exposure to OPs in Californian. 90 controls	Mood scales Motor speed & dexterity Sustained attention Hand-eye coordination RT Digit symbol Visual memory Digit learning	All subjects were exposed to one or more OPs between 1982-1990 and had sought medical help.	Exposed subjects obtained lower scores on a test of sustained attention. Those who were more acutely poisoned also obtained lower scores on tests of psychomotor speed.
Gershon & Shaw 1961	Psychiatric case studies Follow-up study	16 subjects	Psychiatric assessment	Range of occupations. 11 acutely poisoned. Duration of exposure 1.5-10 years.	All 16 reported cognitive impairment. 7 Depressive reactions 5 SZ reactions 1 Fugue 3 cognitive impairment only
Amr et al 1997	Group comparison	380 Egyptian pesticide formulators and applicators 233 controls	Psychiatric Assessment GHQ DSM-III-R	Exposed to a range of pesticides for at least 2 consecutive years.	Increased psychiatric morbidity in exposed subjects, especially dysthymic disorder.

Stallones & Beseler 2002	Postal survey	761 farmers in Colorado			Lower rates of depression than general public, but those with acute poisoning were more likely to be depressed.
Salvi et al 2003	Follow up study	37 tobacco growers 25 then followed up 3 months later	MINI (Psychiatric) ESRS (Parkinsonism) Mini-mental (Cognitive) Word span (Cognitive) Cholinesterase monitoring	1st tests done after 3 months of OP exposure. 2nd tests done after 3 months of no exposure.	Persistant extrapyramidal symptoms after halted OP use. Increased anxiety and depression following exposure.

Studies in individuals with no past history of acute OP poisoning

Please see Table 4 for a summary of this section.

The possibility that chronic low-level exposure to OPs, in doses below that causing acute toxicity may cause ill health is not universally recognised and previous research has yielded inconsistent results. There are several studies of importance in this debate and the populations examined include farmers, pesticide applicators, greenhouse workers and fruit-tree sprayers. However, it is important to note that the OPs used and patterns of exposure may differ greatly amongst these occupational groups and they may not be strictly comparable.

Neuropsychological

Bosma, Boxtel, Ponds et al (2000) examined data from the Maastricht Aging Study, a 3 year prospective cohort study of cognitive ageing in southern Netherlands. 1069 people (age range 50-80 years) were examined at baseline and asked about exposure to certain chemicals including solvents, metals and pesticides. Seventeen people reported frequent exposure to pesticides and at follow-up 35% of them were found to show evidence of mild cognitive dysfunction (assessed by Stroop, verbal fluency, verbal learning and letter digit coding) compared to 11% of an unexposed population. The risk of cognitive impairment was nearly five times higher in exposed individuals. Arable farmers and gardeners were found to have the highest probability of pesticide exposure. Effect modification for confounding variables (e.g. alcohol consumption, smoking, relevant diseases, head trauma etc) did not alter the results. The authors conclude that the results should be interpreted with caution given the small number of individuals in the exposed group and they provide no data regarding severity, frequency and duration of exposure to pesticides making it impossible to determine whether individuals have a history of acute poisoning.

Maizlish, Schenker, Weisskopf et al (1987) studied 46 pesticide applicators in California and compared them with non applicators before and after a work shift. They

found the groups did not differ on tests of memory and RT but the applicators performed more poorly on tests of psychomotor speed and pattern comparison. They concluded that the latter did not relate to OP exposure as they failed to find a correlation between their index of exposure and test scores. However, this assumes a linear effect. Yet again, the lifetime exposure history of subjects is not specified; the duration of exposure prior to testing was only 39 days the inherent toxicity and dose of the pesticide (diazinon) being used was low; the method of pesticide application is more typical of garden and lawn maintenance than commercial pest control or agriculture; and a high level of protective equipment was used by participants (including respirators, face shields, overalls, rubber gloves and boots). Nevertheless, this study suggests that in the short term, low-level exposure (in the absence of any signs of acute poisoning) is not associated with the development of significant neurobehavioural problems, although a couple of abnormalities of unknown significance were identified in the exposed cohort.

Ames, Steenland, Jenkins et al (1995) examined 45 Californian subjects with a prior history of documented cholinesterase inhibition (according to medical supervision records), but with no clinical symptoms of acute poisoning and compared them to controls on the same battery of tests as Steenland et al (1994). They did not find any evidence of neurobehavioural problems in the exposed cohort, whereas in an earlier study, individuals with a history of acute poisoning showed impaired performance on some neurological tests. The authors conclude that neurological sequelae can be prevented by avoiding acute poisoning. However, no information is provided about the duration of time workers have been exposed to pesticides making it impossible to determine whether the findings relate to short or long-term exposure to OPs.

Albers, Berent, Garabrant et al (2004) compared 53 chlorpyrifos manufacturing workers with 60 control subjects whom they examined twice over a period of 1 year. All subjects underwent a neurological examination, general medical examination and completed the mini mental state test (MMSE). Urine and blood tests were used to assess recent exposure and ensure none of the participants had an episode of acute poisoning

over the course of the year. The authors conclude that chronic exposure to chlorpyrifos is not associated with central nervous system dysfunction, although they admit that the results do not preclude the possibility that mixtures of OP compounds might have negative health effects; or that chlorpyrifos might cause subtle neurobehavioural effects which they were unable to detect in a standard neurological examination. Yet again, insufficient information about exposure history is provided; the only test of cognitive ability was the MMSE which is not sensitive to mild or focal cognitive deficits; and subjects were only followed up for one year.

Korsak and Sato (1977) divided 59 male volunteers with varying degrees of occupational exposure to OPs into high and low exposure groups. Impairments were detected in the trail-making part B test and the Bender Visual Motor Gestalt test. EEG differences were also detected. The authors conclude that the left frontal lobe of the brain is particularly vulnerable to OPs. However, many of their subjects were exposed to other chemicals in addition to OPs (e.g. organochlorines and carbamates) making it impossible to determine whether OPs were solely to blame; and detailed information about severity, frequency and duration of exposure to pesticides is not provided, making it impossible to determine whether individuals have a history of acute poisoning.

Rodnitzky, Harvey, Levin et al (1975) studied 23 farmers and commercial pesticide applicators in Iowa who regularly used OP compounds (and had done so within 2 weeks of testing) and compared them with 23 non-exposed farmers. Mean plasma AChE levels were lower in exposed farmers (but within normal limits) but the groups did not differ significantly on tests of memory or RT. However, applicators had higher levels of anxiety. Limitations of this study include the possibility that the control group, who were also farmers, had significant levels of exposure to OPs in the past; the lifetime exposure history of the subjects was not provided; and each subject in the exposed group had used an OP compound within 2 weeks of being tested, making is possible that this study was picking up acute and chronic effects of OPs.

Steenland, Dick, Howell et al (2000) looked at the effects of low-level exposure to an OP pesticide called 'chlorpyrifos' by examining 191 termiticide applicators who had applied this pesticide for an average of 2.4 years, with 189 non exposed controls. All participants underwent an extensive range of tests including clinical examination, urine and blood tests to assess recent exposure and genotype (in regard to paraoxonase), vibrotactile sensitivity, postural sway, manual dexterity, eye-hand coordination, arm/hand tremor, vision and olfaction tests, nerve conduction velocity and cognitive function. The exposed subjects reported more symptoms including memory problems, emotional states, fatigue and loss of muscle strength, but few significant differences were found on quantitative tests. The exposed subjects performed more poorly than controls on pegboard turning tests and some postural sway tests, but there were no significant differences between the exposed and nonexposed groups on most of the cognitive tests. Eight study participants reported a past history of acute poisoning, but only one sought medical help. These men showed a pattern of worse performance on a range of tests including simple RT and continuous performance, when compared to other applicators. The authors conclude that increased symptom reporting in the exposed group is cause for concern, that their neurologic tests may not have been sensitive enough to detect some of the effects of exposure and that there is evidence for delayed effects in subjects with a history of poisoning. This study does not allow for the potential long-term effects of exposure to be determined as the study participants had a relatively short history of exposure to OPs.

Fiedler, Kipen, Kelly-McNeil et al (1997) compared 57 fruit tree sprayers in New Jersey (with no history of acute poisoning resulting in hospitalisation) with controls and found reduced reaction time (although age predicted some of the variance in RT scores) in the fruit tree sprayers, but no other differences between the groups on neuropsychological testing. However, Fiedler et al corrected their data for the influence of reading scores, used to assess premorbid IQ. This may have confounded the results as reading scores may be affected by exposure to OPs.

Farahat, Abdelrasoul, Amr et al (2003) examined 52 Egyptian pesticide applicators during the spraying season and compared them to 50 non-exposed controls (matched for age, years of education and social class). None of the applicators reported an incident of acute poisoning which led to hospitalization. All participants underwent a clinical examination, blood tests to assess recent exposure and psychometric testing. The mean level of serum AChE was significantly lower in exposed subjects but within normal limits and did not relate to performance on psychometric tests. After adjusting for potentially confounding factors (age and education) the performance of the exposed subjects was significantly lower on similarities, digit symbol, digit span, Trails A and B, letter cancellation and the Benton visual retention test. This was related to duration of exposure. The authors conclude that the effects of low to moderate exposure to OPs over a prolonged period of time (10-20 years) may be more wide ranging than previously realized, that workers can exhibit mild symptoms of intoxication without any significant change in blood AChE activity and that psychometric assessment is a useful method for the early detection of chronic effects of OP pesticide exposure.

Srivastava, Gupta, Bihari et al (2000) examined 59 Indian workers recently exposed to different chemicals during the manufacture of 'quinalphos' and 17 control subjects. None of the subjects had a history of acute OP poisoning over the preceding years. All participants underwent a general medical examination, blood tests to assess recent exposure and psychometric testing. Although mean blood AChE levels in the exposed and control groups were not significantly different, exposed subjects reported more symptoms of fatigue and weakness; had a higher prevalence of abnormal plantar and ankle reflex; and lower scores on digit span, digit symbol and Bourdon Weirsma vigilance test. The authors conclude that exposure to OPs can cause CNS damage and that AChE monitoring of chemical plant workers may not be adequate, because OPs may inhibit enzymes other than cholinesterase. The main limitation of this study is the fact that the control group was not matched to the exposed group for level of education and insufficient information about exposure history is provided.

Roldan-Tapia, Parron and Sanchez-Santed (2005) conducted a cross sectional survey of 40 Spanish pesticide applicators who had been employed for 6 months to 30 years, who did not have a history of recorded poisoning events. They were compared to 26 non-exposed controls (matched for age and education). Data were collected at a time of high exposure but serum cholinesterase levels were not significantly different between exposed and non-exposed subjects. A relationship was observed between cumulative exposure and delayed verbal memory, visual memory and anxiety levels. Subjects who had been exposed to pesticides for more than 10 years obtained lower scores on tests of integrative perception and visuo-constructional praxis. The authors conclude that long-term exposure to pesticides can cause neurobehavioural problems.

Studies carried out on Farmers in the UK

The Institute of Occupational Medicine (1999) carried out three phases of research into the relationship between long-term, low-level exposure to OPs and ill health. The first phase of the study was designed to quantify the uptake of OPs in relation to procedural and behavioural aspects of sheep dipping. The results showed that the most important source of exposure was skin contact with concentrated sheep dip, which almost always occurred when the farmer handled concentrate containers in order to dilute the product and replenish the dipping bath. The second phase was a cross-sectional study of exposure to OPs and symptoms of peripheral neuropathy. The third phase of the study is most relevant to the present chapter. A subset of individuals who participated in phase 2 were examined. Equal numbers of individuals with 'no', 'possible' and 'probable/definite' signs of peripheral neuropathy were invited to undergo clinical examination. 74 farmers, were classified into three groups according to whether they had signs of peripheral neuropathy (no, possible, probable/definite neuropathy) and their performance on neuropsychological tests was related to these groupings. Those with neuropathy had poorer mental health. Tests of memory, attention and reaction time were administered. No consistent differences between the groups were found on any of these measures. The IOM acknowledged that their sample size was too small to allow a meaningful analysis of the relationship between cognitive function and exposure history.

Exposure history was not specified or used as a variable in the analysis. The majority of psychometric tests administered were visual and only one verbal memory test was included despite the fact that previous studies suggest verbal functions are affected to a greater degree than visual. The study design is unusual. They have assumed that there should be a relationship between peripheral nerve damage (neuropathy) and central nervous system damage (cognitive function) but this may not be the case, indeed recent studies suggest that PND and CND can be dissociated and that the mechanism underlying each condition may be different (Abou-Donia 2005). Indeed, cognitive impairment may precede other forms of ill health (Bowers and Goodman, 1981). Overall, the value of phase 3 of this study is limited.

Stephens, Spurgeon, Calvert et al (1995), studied the effect of low-level chronic exposure in 146 Farmers who had been exposed to OP sheep dip and compared them with 143 controls. The farmers performed significantly worse than controls on tests of sustained visual attention, syntactic reasoning and speed of information processing. They did not perform worse on tests of memory. They also showed greater vulnerability to psychiatric disorder. The authors concluded that repeated exposure to OPs appears to be associated with subtle changes in the NS, but that these are unlikely to be manifest as clinical symptoms. However, the farmers and controls differed in terms of educational level, alcohol consumption, and first language. Stephens et al used a brief psychometric test battery (digit span, digit symbol, simple RT, syntactic reasoning, serial word list learning and category search), which had been designed for large group research studies and lacked sensitivity in individual cases. It was administered by technicians with no clinical experience and took 1 hour to complete. All or any of these factors may have confounded the results. Finally, Stephens et al did not report whether any of their farmers had a history of dippers flu, making it impossible to determine whether any participants had a history of acute poisoning. Nevertheless, this study raised concern about the effects of chronic exposure to OPs.

Psychiatric

Whether there is an increased rate of psychiatric problems in individuals with a history of low-level exposure to OPs is unclear. An epidemiological study in Australia found that regional usage of OPs was not related to an increased incidence of psychiatric illness as reflected by admissions to psychiatric hospital (Stoller, 1965).

Levin, Rodnitzky, Mick (1976) compared 13 commercial pesticide sprayers and 11 farmers recently exposed to OPs with 24 controls matched for age and education, on tests of personality and, a structured interview and blood cholinesterase activity (ChE). Commercial pesticide applicators had higher rates of anxiety (measured by the Taylor Manifest Anxiety Scale) and lower plasma ChE than controls. However, the plasma ChE levels remained within the normal range. Commercial applicators did not differ from controls in terms of behavioural and somatic symptoms associated with OP toxicity or scores on the Beck Depression Inventory. Farmers did not differ from controls on any variable. Levin et al did not report on the individuals' previous exposure history so it is impossible to determine whether they have suffered any previous episodes of acute poisoning which might account for these findings. Furthermore, exposed subjects had used OP compounds within 2 weeks of assessment. Although drug and alcohol use was recorded, information about past medical history was not. The higher rate of anxiety in the commercial applicators may reflect occupational stress.

Bradwell (1994) describes the case of a 21 year old farm worker who presented with acute organic psychosis, superimposed on a state of chronic anergy and hypersomnia which developed after a 3 year period of regular spraying with organophosphates. He did not wear protective clothing whilst doing this. Initially he developed chronic fatigue, hypersomnia, paraesthesia in his arms, skin blisters, sweating and headaches. Subsequently, he had three nights when he was agitated, disorientated and appeared to be having visual hallucinations. His family reported gradual recovery over 4 months, but then a sudden deterioration in functioning after he consumed 2 pints of beer. He was admitted to a psychiatric hospital and treated with psychotropic medication. He

improved a little, but deteriorated after coming into contact with organophosphate compounds when he returned to his father's farm. EEG and CT brain scan were abnormal and he was diagnosed as having suffered a cerebral encephalopathic illness secondary to imbalance of neurotransmission caused by OP poisoning.

Ahmed and Davies have published several reports concerning neuropsychiatric abnormalities seen in individuals with a history of exposure to OPs. In 1997 they described a neuropsychiatric syndrome (referred to as COPIDN) which they observed in 26 out of 33 clinical cases referred to them for an opinion about the possible relationship between ill health and exposure to OPs. COPIND comprises 10 symptoms the cardinal ones being mood instability, suicidal thinking, cognitive impairment, language disorder, inability to sustain muscular power, alcohol intolerance and olfactory hypersensitivity. After taking into account individuals' medical histories, the authors conclude that the most likely explanation for these symptoms is OP exposure. However, they do not give details about the individuals' exposure histories, other than to state that many suffered episodes of dippers flu. This may indicate that these individuals have a history of acute OP poisoning and should not be included in this section on low-level exposure. Furthermore, they did not use a control group.

In 1999 Davies, Ahmed and Freer report the results of 2 postal surveys in which they attempt to overcome the methodological difficulties associated with their previous study. In study 1 they compared 127 exposed and 43 non-exposed farmers (randomly selected) and found increased reporting of COPIND symptoms in exposed farmers compared to unexposed farmers. In study 2 they examined the symptom profiles of 215 individuals with medically unexplained illnesses which they attribute to OP exposure. Significant similarities in symptom profiles were observed amongst individuals from different occupational groups whose common factor is OP exposure. However, they do not report past medical history, making it difficult to determine whether these individuals have made an attribution error. The response rate in study 1 was low (44.6%) which could indicate a bias in that those individuals who attribute their difficulties to OP exposure

may be more likely to return the questionnaire. Finally, detailed exposure history is lacking and it may be that, as above, many of these individuals have a history of acute OP poisoning.

In 2000 Davies, Ahmed and Freer present a detailed case study of a farmer in his forties who had been regularly exposed to OP sheep dips for 15 years. He complained of mood swings (depression, irritability and anger, but not mania). His past medical history was unremarkable. He had a history of dippers flu. He was diagnosed as suffering from COPIND. Six months later he shot himself. Post mortem revealed extensive peripheral neuropathy consistent with OP poisoning. Yet again, this is probably an example of the chronic effects of repeated episodes of acute poisoning.

Malmberg et al (1999) identified 82 individuals who had committed suicide between 1991 and 1993. They gathered information from GP and inquest records and where possible interviewed relatives/friends to examine the circumstances and motives behind their deaths. 69% of the sample was working at the time of death – the remainder had stopped working due to ill health or retired several years before their death. Most of the data presented relates to those still in employment at the time of death. Problems faced by farmers in the year before death included mental illness (70%, mainly depression), physical illness (52%) and financial (21%) or occupational worries (44%). They conclude that suicide is unlikely to be related to OP exposure as it was not commented on in GP or inquest records. However, this is not surprising as many medical personnel fail to consider OP poisoning as a possible diagnosis. Malmberg et al also points out that sheep farmers were not over-represented among the suicides, but fails to acknowledge that many other farmers (e.g. cattle and arable) also use OPs. They have limited data on those who retired or stopped working on ill health grounds, but do not explain why. Neither do they consider the possibility that those who are ill may have been affected by OPs. With such limited information, it is impossible to draw any valid conclusions about the relationship between ill health, suicide and OP exposure from this study population.

Summary

It is still a matter of controversy whether low-level exposure can result in chronic ill health. Some studies seem to suggest that at least in the short term, if acute exposure can be avoided, significant neurological sequelae can be prevented (Maizlish et al, 1987; Ames et al, 1995; Fiedler et al, 1997; Steenland et al, 2000). Other studies suggest that low-level exposure over a prolonged period of time can cause neurobehavioural problems (Stephens et al, 1995; Srivastava et al, 2000; Roldan-Tapia et al, 2005; Farahat et al, 2003). Inconsistencies among studies are likely to have been caused by inadequate assessment of exposure history and variations in study methodology which make direct comparison of results impossible. There is no agreed definition of acute/chronic or high-/low-level exposure and the majority of studies fail to provide sufficient information about exposure history to reassure the reader that individuals with a history of acute poisoning have been excluded from studies purporting to be examining individuals with a history of low-level exposure. Furthermore, using 'diagnosed/recorded poisoning' as the definition of acute exposure may result in individuals with a history of undocumented mild toxicity being misclassified and assigned to the wrong groups. There is an assumption that level of exposure is the critically relevant biological variable and this leads to the expectation that linear dose-response relationships between health and exposure history will be found. Future studies should consider the possibility that (1) a linear relationship between level of exposure and health does not exist, and (2) the considerable number of variables which can influence toxicity should be taken into account (see Chapter Two).

Aims of the present study

The present study was designed to establish whether agricultural workers with a history of prolonged exposure to OPs show evidence of cognitive impairment. This is a detailed, small group study which attempts to overcome some of the methodological weaknesses of previous research, in particular, detailed information concerning exposure history is obtained, a comprehensive and clinically sensitive psychometric test battery was used

(similar to what would be used in clinical practice) and past medical and psychiatric history were taken into account to exclude other possible causes of ill health and to ensure participants had not made an attribution error. Specific aims and hypotheses are detailed in Chapter Four.

Table 4: Studies of individuals with NO past history of acute OP poisoning.

Reference	Type of study	Subjects	Tests/functions	Exposure	Result
Bosma et al 2000	3 year prospective study in the Netherlands of 1069 people involved in a study of ageing	17 subjects reported exposure to OPs	Stroop Verbal learning Word fluency Letter-digit coding	Detailed information on severity, frequency, duration of exposure not provided.	35% showed mild cognitive impairment compared to 11% unexposed subjects. Farmers & gardeners most at risk.
Maizlish et al 1987	Pre and post exposure during a work shift	46 pesticide applicators 56 controls	Choice RT Digit symbol Hand-eye coordination Vocabulary Perception Finger tapping	Duration of exposure was short (39 days). Lifetime exposure not specified. Lots of protective clothing used.	Applicators performed more poorly on tests of psychomotor speed and pattern comparison, but not RT or memory.
Ames et al 1995	Follow-up study of workers who had been removed from exposure because of blood test results	45 Californian 90 controls	Mood scales Motor speed & dexterity Sustained attention Hand-eye coordination RT Digit symbol Visual memory Digit learning	Evidence of cholinesterase inhibition, but no clinical symptoms.	No evidence of neurobehavioural problems. Preventing acute poisoning may prevent neurological sequelae.
Albers et al 2004	Group comparison Tested twice over a period of 1 year	53 OP manufacturers 60 controls in similar occupation	Overt questionnaires of cognition and neurologic complaints. MMSE Nerve function Coordination Motor & reflex skills	Exposure to chlorpyrifos. Detailed cumulative exposure estimates were made using work records and questionnaire. Evidence of cholinesterase inhibition.	No evidence of CNS dysfunction.

Korsak & Sato 1979	Group comparison	59 volunteers, split into high vs low exposure groups	Manual dexterity Memory Trails B Bender VMG test Zung Depression scale	Lifetime exposure index calculated. Volunteers had also been exposed to hydrochlorines and carbamates. Plasma AChE levels measured to exclude participants with acute exposure.	Groups differed on Trail-making-B and Bender visual motor gestalt tests.
Rodnitzky et al 1975	Group comparison	23 farmers & pesticide applicators who had used OPs in the last 2 weeks on crops 23 farmers with no recent exposure	Verbal recall (Peterson) RT Sentence repetition Proprioception	Controls may have been exposed in the past.	No differences on tests of memory or RT. Applicators have higher levels of anxiety and lower plasma cholinesterase (but within normal limits).
Steenland et al 2000	Group comparison	191 exposed termiticide applicators 189 controls	Vocab Mood scales Digit span Continuous performance Simple RT Digit symbol Pattern memory Vibrotactile test Arm/hand tremor Postural sway Manual dexterity Hand-eye coordination Vision Olfaction Nerve conduction	Termiticide applicators who had at least one year's exposure to pesticides. Urine TCP levels were taken. PON1 was also looked at.	The exposed group reported more problems of memory, emotional state, fatigue and muscle strength, but few differences were found on quantitative tests. The exposed group were impaired on pegboard turning and some postural sway tests, but were not significantly different from controls on most cognitive tests. 8 subjects who had a history acute exposure showed impaired RT & continuous performance.

Fiedler et al 1997	Group comparison Investigators blind to exposure status	57 fruit tree sprayers 42 age matched controls Controls and subjects had different reading scores (and levels of education) so reading score was used as a covariate in the analysis	RT Trails A&B Manual dexterity Hand-eye coordination Digit symbol Digit span Information CVLT WMS-R visual reprod. Visual recognition Stroop Semantic fluency Token Test MMPI-2	Lifetime exposure index calculated. Subjects with a history of acute poisoning, requiring medical intervention excluded.	Exposed subjects had slower RTs.
Farahat et al 2005	Group comparison	52 Egyptian crop sprayers 50 matched controls (age, class, education)	General medical exam Nerve function Motor & reflex abilities Sensory measurements Similarities Digit span Digit symbol Trails A&B Block design Serial addition Letter cancellation Benton visual retention Story recall	Involved in extensive cotton spraying for the last 3 years. Evidence of some cholinesterase inhibition in the exposed group, but within normal levels. No acute poisoning that lead to hospitalization was reported.	After adjusting for age & education, they found exposed subjects performed significantly lower on similarities, digit symbol, digit span, Trails A&B, letter cancellation and the Benton visual retention test. This was related to duration of exposure.
Srivastava et al 2000	Group comparison	59 Indian quinalphos manufacturers 17 controls	General medical exam Digit span Digit symbol BW vigilance test	AChE levels were taken, but no difference between groups. No symptoms of acute poisoning were reported over preceding years.	Exposed subjects reported more neurobehavioural problems (e.g. weakness & fatigure), exhibited some abnormal reflexes and scored lower on digit span, digit symbol and BW vigilance test.

Roldan-Tapia et al 2005	Group comparison	40 Spanish greenhouse workers 26 controls	Stroop Letter cancellation Trail making Digit vigilance Picture completion Similarities Digit span Verbal learning Complex figure Benton visual retention Logical memory test Visual discrimination Block design Naming Ideomotor apraxia tests Ideational apraxia tests Mood scales	Record of previous pesticide exposure was established through a structured questionnaire. Serum cholinesterase levels were taken (they were not significantly different to controls). No recorded poisoning events.	A relationship was observed between cumulative exposure and delayed verbal memory, visual memory and anxiety levels. Subjects who had been exposed to pesticides for more than 10 years obtained lower scores on tests of integrative perception and visuo-constructional praxis.
IOM study, 1999	Group comparison Investigators blind to exposure status	74 farmers classified into 3 groups according to whether they had signs of periperal neuropathy	NART Rey AVLT HAD, GHQ *CANTAB* RT Visual search Visual memory	Detailed information not provided. Exposure index calculated.	Those with neuropathy had poorer mental health, but no consistent differences in cognitive function observed between groups.
Stephens et al 1995	Group comparison (Random selection)	146 sheep dippers 143 controls	Digit span Digit symbol RT Syntactic reasoning Verbal list learning Category search	Urine tests used to exclude subjects with recent exposure to Ops. Detailed information about exposure not provided, but index calculated	Farmers had lower scores on tests of information-processing speed and sustained attention, but not memory. They also have increased rates of psychiatric morbidity.

Levin et al 1976	Group comparison Investigators blind to exposure status	24 farmers & pesticide applicators 24 farmers with no recent exposure	Taylot Manifest Anxiety Scale Beck Depression Inventory	Used OP compounds 2 weeks prior to assessment. Pesticide applicators had lower plasma cholinesterase levels (but within normal limits) Controls may have been exposed in the past.	Applicators had higher rates of anxiety. Groups did not differ in terms of somatic and behavioural symptoms of OP poisoning or on Beck Depression Inventory scores.
Ahmed & Davies 1997	Clinical cases referred to psychiatry for an opinion about the relationship between OP exposure and ill health	33 clinical cases	Psychiatric assessment	Detailed information about exposure not given, just occupational groups; sheep dipping, arable farming, gardening, pest controllers etc.	26 show a constellation of specific neuropsychiatric abnormalities (COPIND).
Davies & Ahmed 1999	Postal survey	127 exposed farmers 43 non-exposed	Symptom questionnaire designed by authors.	Detailed information about exposure not given, just occupational groups; sheep dipping, arable farming, gardening, pest controllers etc.	Increased rate of COPIND in exposed farmers.
Malmerg et al 1999	Analysis of GP and inquest records pertaining to farmers who had committed suicide. Also interviewed relatives/friends	82 suicide victims		No information about exposure history provided.	70% were depressed and 52% ill prior to death. Suicide is unlikely to relate to OP exposure as it is not commented on in records, sheep dippers are not over-represented in this group.

CHAPTER FOUR: METHOD

Study design

This is a cross sectional, between subjects study comparing neuropsychological performance in 25 agricultural workers who were exposed to organophosphate pesticides in the course of their work, with 22 non-exposed healthy volunteers (controls), matched to the exposed group for age, gender, years spent in education and estimated premorbid ability/intelligence.

Ethical Approval and Informed Consent

Ethical Approval for this research project was granted by the joint UCL/UCLH committee on the Ethics of Human Research, Committee A and written informed consent was obtained from all study participants (see Appendix A).

Overview

The author was contacted by a firm of solicitors in May 1998 and invited to become involved in a multi-disciplinary study of agricultural workers who report ill health which they attribute to OP poisoning. The solicitors were acting for the claimants and were seeking evidence that their client's health had been adversely affected by exposure to organophosphate compounds. The solicitors were recruiting a team of medical experts both from the U.K. and abroad to collate evidence needed to prove causation in these cases. Unusually, they had obtained permission and funding from the legal services commission to carry out a research study / group analysis (referred to as the 'medical pilot study') in addition to preparing reports on individual claimants. Individual claimants gave consent for their data to be included in the pilot study. The solicitors required a clinical neuropsychologist to carry out psychometric testing and to (1) prepare reports on individual clients and (2) become part of the medical pilot study and undertake groups anlayses. The author agreed to assist with this study and devised a battery of psychometric tests to be used in the assessment of each client. Over the next three years the author assessed over twenty individuals. A colleague, Dr Julia Clark,

Clinical Neuropsychologist assessed a further twelve clients during a brief period of time when the author was overseas.

General Aims

(1) To establish whether agricultural workers with a history of prolonged exposure to OPs show evidence of cognitive impairment.

(2) To examine the nature and extent of any cognitive deficits identified.

(3) To determine whether the pattern of cognitive deficit relates to the frequency or duration of exposure to OPs.

Specific hypotheses

In addition, this study will examine the following hypotheses:

(1) Participants will show a similar pattern of cognitive and emotional deficits as that reported in earlier studies of individuals with a history of chronic low-level exposure to OPs. They will perform poorly on tests of information-processing speed, psychomotor speed and sustained attention. In addition, they will have high rates of emotional distress.

(2) Some, if not all, participants may have been erroneously classified as having low-level exposure when it would be more appropriate to classify then as having had previous episodes of acute poisoning. Participants will be asked about episodes of possible acute poisoning (i.e. dippers flu) for which they did not seek medical attention. As far as the author is aware, this has not been done before.

(3) Those farmers with a history of dippers flu will show a similar pattern of deficits to that seen in individuals with a history of one or more episodes of acute poisoning. In addition to the deficits described in (1) above, they will show impairments of memory, language and executive function.

Participants – Exposed Group

Thirty-three individuals with a history of exposure to OPs were assessed by the author and Julia Clark and all participants were involved in litigation. They are a self-selected sample and therefore it is unclear to what extent their results can be considered representative of the general population of agricultural workers.

The vast majority (twenty one) were farm workers who had been exposed to OPs over a number of years as a result of sheep dipping. Two people were government officials (animal health inspectors) who supervised sheep dipping; four people spent many days each year involved in crop spraying, in addition to sheep dipping; two people were only involved in crop spraying; one individual was exposed to OPs when he transported OP concentrate to farms, one individual was exposed to OPs after observing his parents sheep dipping over a number of years; one individual was exposed to OPs through the course of his work as a plant technician; and one person was exposed to OPs whilst treating pet animals with flea powder.

Table 5: Participants' occupational groups.

Occupation	Number of participants
Farmers involved in sheep dipping	21
Animal Health Inspectors involved in sheep dipping	2
An individual who observed sheep dipping on a regular basis throughout childhood	1
An individual who transported sheep dip concentrate	1
Crop spraying + sheep dipping	4
Crop spraying only	2
A plant research technician	1
A person who treated pet animals with flea-powder	1

Exclusion Criteria

Individuals were excluded from further analysis if they had a medical or psychiatric history which might otherwise account for any cognitive or emotional problems identified at interview, for example previous neurological damage (congenital or acquired), serious systemic disease, metabolic or endocrine disorder, psychiatric illness, oxygen deprivation, exposure to other toxins, alcohol or drug abuse.

Six individuals with a history of neurological or psychological illness prior to exposure were excluded from further analysis and another two individuals were excluded for the following reasons (1) one person suffered a traumatic brain injury following exposure to OPs (2) one individual received ECT treatment as their mood state deteriorated to a significant degree following exposure to OPs. These cases were excluded because it would be impossible to determine the extent to which any identified cognitive deficits were a direct result of OP poisoning as opposed to further neurological damage (see Table 6).

Table 6: Reasons for exclusion from further analysis

ID	**Previous neurological or psychological illness**
1	Traumatic brain injury following an assault resulting in fronto-temporal lobe damage
2	Subarachnoid haemorrhage (anterior communicating artery)
3	Neurological damage following a diving accident
4	Traumatic brain injury, exposure to other toxins, depression and ECT treatment
5	Participation in chemical warfare experiments
6	A history of chronic fatigue syndrome, psychological disorder, somatic complaints and a poor temporal relationship between symptoms and exposure to OPs
	Further neurological damage sustained after exposure to OPs
7	Severe depression and ECT treatment
8	Traumatic brain injury following exposure resulting in left temporal/parietal contusions

Remaining Sample – Exposed Group

Twenty five individuals were included in the final group analysis (mean age 48 years; range 20-65) and all bar one individual was male. The mean number of years spent in education was 12 years and the mean Wechsler Adult Intelligence Scale-Revised, Full Scale IQ was 102 points in this group.

Participants – Control Group

Finding a suitable control group for pesticide-exposed agricultural workers is difficult. Most farming involves the use of pesticides, so it is extremely difficult to identify agricultural workers who have not been exposed to pesticides to act as controls. In this study, healthy volunteers who did not work in the farming industry were selected to act as controls. Twenty four individuals were recruited from local job centres within London and newspaper advertisement. Two were excluded from further analysis as they had a medical or psychiatric condition which might otherwise affect cognitive function (e.g. previous cancer treatment, drug and alcohol problems), leaving a final sample of 22 individuals who were matched to the exposed group on gender, age, years spent in education and level of intelligence. Twenty one individuals were male and one was female. Their mean age was 46 years (range 24-65) and the mean number of years spent in education was 12 years and this is not significantly different from that seen in the exposed group ($t=0.918$, $p=.36$; $U=219.5$, $p=.21$). The mean Wechsler Adult Intelligence Scale-Revised, Full Scale IQ in the control group was 109 points and this is not significantly different from that seen in the exposed group ($t = 1.619$, $p<.112$).

Table 7: Characteristics of study participants

Subjects	Number	Mean Age	Mean years in education	Mean WAIS-R Full Scale IQ
Agricultural workers	25	48 years	12 years	102
Healthy Controls	22	46 years	12 years	109

Procedure – Exposed Group

Individuals were referred directly to the author or Julia Clark. Prior to assessment, the referring solicitor sent a bundle of documents which contained the following information:

(1) A detailed witness statement regarding exposure history (including documentary proof, such as receipts regarding the purchase of OPs, work diaries etc).

(2) Copies of each individual's general medical notes (complete set) and any relevant hospital records.

(3) Copies of any other medico-legal reports prepared to date e.g. psychiatric and neurological reports.

Each individual underwent a clinical interview followed by a detailed neuropsychological assessment which took the best part of a day to complete. Participants were offered the opportunity for breaks during the day.

Content of the Clinical Interview

The following information was obtained during the clinical interview and whenever possible a relative/carer was interviewed as well to obtain corroborating evidence.

Table 8: Content of the Clinical Interview

Clinical Interview
Developmental and social history
Educational and occupational background
Past medical and psychiatric history; alcohol, drug and medication use
Recent stressful life events (e.g. bereavement, divorce – see Appendix)
Exposure history (see below)
Onset of physical/psychological problems and their temporal relationship with exposure, plus their evolution over time
The nature of any medical treatment provided
Current symptoms/problems (physical, emotional, cognitive)
Impact on daily life
Mood state.

Exposure history

Each participant was interviewed in depth about their exposure history and questionnaires were designed to act as prompts for the author and Julia Clark. The author designed the exposure questionnaire following a review of the literature and discussion with agricultural workers and medical colleagues about the exposure variables which are likely to be important (see Appendix). To summarize, individuals were asked to specify when they began working with OPs, in what capacity, their level of exposure in terms of frequency and duration, the use of protective clothing, their involvement in high risk activities such as diluting concentrate, their use of other agricultural chemicals, the onset of their physical/psychological problems and the temporal relationship with exposure to OPs, and whether or not they had a history of acute poisoning or dippers flu. In addition to obtaining information about specific aspects of exposure an overall exposure index was calculated for each participant based on the number of days per year spent using OPs (this varied according to government directives, please see Chapter One) multiplied by the number of years spent using OPs. Some workers were exposed to OPs during a range of different activities (e.g. sheep

dipping, crop spraying, treating cattle for warble-fly), all of which were taken into account when calculating the overall lifetime exposure index.

Procedure – Control Group

Each individual underwent a shortened version of the clinical interview described above followed by the same detailed neuropsychological assessment undertaken by the exposed group (see below).

Psychometric Assessment

All participants underwent detailed psychometric testing. Only well known, reliable and clinically sensitive measures were selected for inclusion in the test battery. As was mentioned in Chapter Three, previous studies have often used very limited test batteries which (1) do not assess all classes of cognitive function and/or (2) may lack sensitivity as researchers have extracted subtests from larger test batteries (e.g. digit symbol) or substituted laboratory tasks which are not routinely used in clinical practice. For the present study, tests were selected which would assess a broad range of cognitive functions including premorbid and current IQ, language skills, memory functioning (verbal and visual), information-processing speed, executive function and visuo-perceptual ability. A test of incomplete effort was also included in the battery since all of the participants in the exposed group were involved in litigation and may have a financial incentive to perform poorly on psychometric tests. Finally, emotional state was assessed using the Hospital Anxiety and Depression Scale.

Measures

Premorbid Ability

The National Adult Reading Test (Nelson, 1982) was used to assess premorbid ability. This estimate of IQ is relatively resistant to organic brain damage and correlates highly with Wechsler Adult Intelligence Scale-Revised (WAIS-R) IQ scores in healthy adults (Crawford, 1989). However, there are two circumstances when the NART may underestimate an individual's premorbid IQ: (1) when an individual has a history of

disrupted schooling and this has affected their reading ability; (2) when an individual's language ability has been affected by organic brain damage. In these circumstances, clinicians compare the NART score with the scores an individual obtained on other tests which are thought to be fairly resistant to organic brain damage, such as the vocabulary subtest of the WAIS-R.

Current IQ

The Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981) was used to assess current intellectual functioning. This is a well known and highly validated test which most clinical neuropsychologists include in their test batteries (Lezak, 2004). The WAIS-R is composed of eleven subtests. Six measure verbal skills and are predominantly, but not entirely, associated with the functioning of the left hemisphere of the brain. Five measure non-verbal skills and are associated with right hemisphere functioning. In addition, Ravens standard progressive matrices test was administered (Raven, Court and Raven, 1996) as it is thought to be relatively unaffected by cultural factors such as previous academic experience.

Memory

The Adult Memory and Information Processing Test Battery (AMIPB: Coughlan and Hollows, 1985) was used to assess visual and verbal memory. There are four subtests which assess memory functioning; a list learning task, prose recall, a design learning task and immediate and delayed recall of a complex figure. This test was standardized on people who were born and educated in the UK unlike the slightly better known Wechsler Memory Scale-Revised (WMS-R) which was standardised on American subjects and is more frequently used in research studies. Another advantage of the AMIPB is that it takes less time to administer than the WMS-R.

Language

The Graded Naming Test (McKenna and Warrington, 1983) was administered to assess naming ability as it is sensitive to mild degrees of anomia. A verbal fluency test (FAS: Borkowski et al, 1967) was used to assess expressive language and frontal lobe function.

Mental Flexibility (executive/frontal lobe function)

The Stroop test (Trenerry et al, 1988) was included as a measure of mental flexibility and frontal lobe function. Performance on the following tests was also taken into account when considering the efficiency of frontal lobe function: similarities, picture arrangement and digit span subtests from the WAIS-R, verbal fluency and Trails A and B scores.

Information Processing Speed Tests

AMIPB Information Processing Speed Tests (Coughlan and Hollows, 1985) were included in the test battery as separate measures of mental and motor speed can be calculated from this test. The better known Trail Making Test A and B (Spreen and Strauss, 1991) was also included in the battery.

Perception

Benton Line Orientation Test and the Benton Face Recognition Test (Benton et al, 1994) were included to assess visuo-perceptual skill. Both tests are sensitive to posterior right hemisphere lesions.

Testing for Incomplete Effort

Since all of the agricultural workers who participated in this study were involved in litigation, the possibility that some individuals may have simulated or exaggerated their deficits for financial gain was considered. Performance on a number of tests was examined to look for evidence of inconsistency, and/or suboptimal effort. Clinicians rely on a range of methods to determine the extent to which a patient's symptoms have an organic basis including (1) consistency of performance (2) whether the pattern of

impairment is similar to that seen in patients with organic conditions (3) the severity of impairment and whether scores fall below chance levels (4) comparisons between the performance curves of bona fide patients and those suspected of malingering, and (5) an understanding of the patient's background. In addition, various tests are considered to be sensitive to faking, some have been specifically designed to detect malingering (e.g. Rey's test of Malingering). However, research has shown that the ability of skilled clinicians to detect feigned neuropsychological deficits when provided with standard data from the WAIS-R and WMS-R seldom exceed chance levels and that tests designed to assess malingering often lack sensitivity (Rogers et al, 1993). A combination of strategies produces the highest classification rates.

A range of techniques were employed to assess the validity of test performance in the exposed cohort such as examining the consistency of performance across trials on Ravens Matrices and the Trails A and B tests, examining the quality of performance on memory tests (e.g. the presence/absence of primacy and recency effects, comparing digit span scores with performance on immediate memory trials from list learning tasks, etc - and Rey's Test of malingering (15 item – see Appendix C: Lezak, 2004) was included in the test battery to assess the likely validity of each participant's test performance. This test consists of 15 items (letters, numbers and shapes) displayed in five rows of three items. The principle underlying the test is that anyone who is not significantly impaired can recall at least three of the five character sets. However caution is advised in interpreting the results as evidence of malingering, in patients who show a significant level of cognitive impairment. Many patients who are intellectually impaired fail this test, indeed poor performance is common in brain injured patients (Rogers et al, 1993) and for this reason it is not suitable for use with mentally dull individuals or those with neurological disease (Lezak, 2004). Rey's test was included in the current battery because it was assumed, given the findings of previous research concerning the effects of low-level exposure to OPs, that participants were unlikely to suffer from significant levels of impairment. When Rey's test has been administered to individuals who are subsequently found to have significant levels of cognitive impairment, Lezak (1995)

recommends taking the quality of performance into account in addition to the overall score. Malingerers are more likely to deny recall and make errors of omission, whilst patients who fail the test as a result of significant cognitive impairment are more likely to make perseverative or sequencing errors. When patients are significantly impaired, it is inadvisable to rely on the results of one test and its cutting score when evaluating performance validity (Pankratz, 1988). Indeed research suggests that a combination of strategies produces the best classification rates, particularly those which include an examination of performance curves.

Mood State and Life Events

The Hospital Anxiety and Depression Scale (HAD: Snaith and Zigmund, 1983) was used to assess participants' level of anxiety and depression at the time of testing. It is a well known, reliable and valid self assessment scale specifically designed to detect anxiety and depression amongst medical patients. The scale excludes items which might relate to the somatization of mood or physical illness such as loss of appetite etc. A life events checklist was also included (see Appendix C: Holmes and Rahe, 1967) to act as a prompt for the author and Julia Clark when interviewing participants about recent stressful life events.

Table 9: Psychometric tests included in the neuropsychological battery.

Psychometric Test Battery
National Adult Reading Test (NART)
Wechsler Adult Intelligence Scale-Revised (WAIS-R)
Coughlan and Hollows Memory and Information-Processing Speed Battery (AMIPB)
Graded Naming Test (McKenna and Warrington)
Stroop Test
Borkowski Verbal Fluency (FAS)
Benton Line Orientation Test
Benton Face Recognition Test
Trail-Making A and B
Rey's test of malingering (15 item)
Hospital Anxiety and Depression Scale (HAD)
Life Events Checklist (Holmes and Rahe)

Data Analysis

Section 1

In section 1, participants' exposure histories are described followed by a brief review of the physical symptoms associated with exposure and the results of various medical tests aimed at establishing the aetiology of these complaints.

Section 2

In section 2 differences in performance between the exposed and control cohorts on psychometric testing was compared using Mann Whitney U and t-tests. The normality of distribution around the mean was analysed for all variables. It was found that a number of variables exhibited unacceptable skewness and/or kurtosis for parametric statistical analyses. Square root transformations and/or natural logarithm transformations corrected these distributions in some cases, but several variables remained abnormally distributed and had to be analysed using non-parametric tests (see Table 10).

Table 10: Variables which were abnormally distributed

Variable	Square root transformation	Natural logarithm transformation	Abnormal distribution
Demographic IQ	X		
AMIPB: Information processing subtests	X		
Face recognition		X	
Education			X
WAIS-R: Block Design			X
Naming			X
AMIPB: complex figure			X
Stroop			X
Trails A and B			X

Section 3

Pearson product moment correlations and linear regression were used to establish whether there is a relationship between cognitive function and exposure history. Four indices of exposure were used in the analyses: (1) the total number of years spent working with OPs (duration of exposure); (2) a lifetime exposure index (no years of exposure x no days per year of exposure); (3) the number of years which had elapsed between the last exposure and the current assessment; (4) the number of years which had elapsed since the onset of ill health (duration of ill health). Results from the following psychometric tests were included in the analyses: Story recall (a test of both information-processing speed and memory); list learning (a test of verbal learning); digit span (a test of working memory and attention); digit symbol (a test of psychomotor speed); AMIPB motor and mental speed (tests of information-processing speed); verbal fluency; Stroop (tests of executive function); and WAIS-R Verbal IQ scores. These tests were selected following a review of the literature concerning the cognitive deficits associated with exposure to OPs and the findings of the present study, reported in section 2 (Bosma et al, 2000; Roldan-Tapia et al, 2005; Srivastava et al, 2000; Farahat et al, 2003; Korsak & Sato, 1979; Maizlish et al 1987; Fiedler et al, 1997; Stephens et al, 1995). Results from the remaining psychometric tests were not included in the analyses to reduce the risk of Type 1 errors occurring as a result of multiple comparisons. A Type 1 error involves concluding that a meaningful relationship (or difference) exists between

variables, when it actually occurred by chance (please see Chapter Six for further discussion of this issue and how it relates to this study).

Data were tested for normality and homogeneity of variance to determine whether parametric or non parametric tests should be used. The distribution of two key exposure variables differed significantly from normality (total exposure index and the number of years since the last exposure), as did one of the psychological variables used in the analysis (Coughlan and Hollows information processing speed scores). Square root transformations were computed to correct for this so that the data could be analysed using parametric statistics.

Statistical Power

Finding comparable studies in order to calculate power calculations proved difficult. As was mentioned in Chapter Three, studies have utilized different methodological designs, populations, psychometric tests and control groups making direct comparisons difficult. Studies by Stephens (1995) and Rosenstock (1991) found moderate effect sizes (0 .51 -0 .59) between cognitive function and exposure history indicating that future studies would require a sample size of sixty two individuals to have 80% power to detect a relationship of this magnitude between neuropsychological functioning and exposure history. However, the study participants in Stephens and Rosenstock studies differed in important ways to the participants involved in the present study. Whilst both studies used similar psychometric test batteries, they examined individuals who were fit enough to be in employment and may have excluded individuals with more serious health problems who may have retired or changed occupation. Seventeen individuals in the current study have retired on ill health grounds and the remainder have significantly reduced their working activities. Given the level of disability in this cohort, large effect sizes were anticipated between exposure history and cognitive function.

CHAPTER FIVE: RESULTS

Overview

First the participants and their exposure histories will be described followed by the nature and extent of neurobehavioural problems identified in this cohort. The relationship between exposure history and cognitive impairment will be discussed and finally the relationship between the study hypotheses and the results will be addressed. This study is primarily concerned with the neuropsychological sequelae of OP exposure, but a brief review is provided of the physical symptoms associated with exposure and the results of various medical tests aimed at establishing the aetiology of these complaints.

SECTION 1 : Participants and Exposure History (descriptive information)

Demographic information

Table 11 presents participants' demographic information. The exposed and control groups were well matched for age, years spent in education, premorbid IQ when estimated from demographic variables (demographic IQ) and current level of intellectual functioning (WAIS-R, full scale IQ). They were not well matched on premorbid ability estimated from reading ability using the National Adult Reading Test (NART). While NART is a valid test of premorbid functioning for the majority of people, there are some groups for which it is not accurate (e.g. those with language disturbances). To establish whether or not NART is a suitable measure, Crawford et al (1990) developed a regression equation that will predict NART performance from demographic information. A negative discrepancy of more than 9 points between predicted and obtained scores suggests impaired NART performance and is therefore unlikely to provide a valid estimate of an individual's premorbid cognitive ability. This was the case for 12 of our exposed cohort. and suggests that language ability is poorer than expected in exposed subjects. When there is reason to suspect that NART performance may have been impaired by organic brain damage, Crawford and Allan (1997)

recommend the use of demographic variables to predict premorbid ability and have devised a formula which predicts WAIS-R IQ from age, years spent in education and social class (Crawford and Allan 1997). The equation is as follows:

Predicted WAIS-R FSIQ = 87.14 – 5.21(social class) + 0.18(age) + 1.78(education)

Age was recorded in years. Education was calculated from their school or university leaving age and was also recorded in years. Social class was determined by each study participant's occupation and was coded using the Office of Population Censuses and Surveys (1980). Retired and unemployed participants were coded by their previous main occupation.

Table 11: Characteristics of farmers and controls

Characteristics	Farmers (n=25)	Controls (n=22)
Mean age (SD) in years	48 (10.6)	46 (10.9)
Mean educational level (SD)	12 (2.8)	12 (2.1)
Mean NART IQ*	96 (13.4)	111 (6.7)
Mean Demographic IQ	102 (9.6)	102 (5.5)
Mean WAIS-R full scale IQ	102 (13.6)	109 (12.3)

*significantly different between groups per unrelated t test

Table 12: Summary exposure history

ID	Age	Occu. now	Occu. Then	Years of exposure	Exposure Index	Start work	Ill health starts	Stop work
1	59	semi	Dip	18	50	1976	1991	1994
2	53	retire	Dip/sup	10	600	1982	1988	1992
3	43	semi	Dip/trad	31	441	1966	1994	1997
4	35	semi	Dip/con	10	378	1984	1993	1994
5	48	retire	Dip	14	168	1972 (80)	1993	1994
6	39	retire	Spray	5	400	1982	1982	1987
7	51	retire	Spray/D	30	287	1966	1993	1996
8	20	study	Dip/obs	5	12	1987	1993	1992
9	53	retire	Dip/sup	19	840	1979	1990	1998
10	50	retire	Spray/D	25	2555	1972	1997	1997
11	56	retire	Dip	20	200	1978	1989	1998
12	42	semi	Dip/con	18	649	1976	1997	1994
13	57	retire	Spray/D	32	3028	1965	1980	1997
14	56	retire	Dip	23	100	1950 (73)	1987	1996
15	32	retire	Dip	4	15	1990	1993	1994
16	55	retire	Dip	7	14	1985	1991	1992
17	56	retire	Dip	16	64	1980	1993	1996
18	53	retire	Dip	6	210	1983	1987	1989
19	57	retire	Dip	3	12	1957 (84)	1987	1987
20	65	retire	Dip	8	30	1984	1992	1992
21	52	semi	Spray	19	781	1960 (74)	1975	1993
22	30	retire	Dip	8	80	1985	1989	1993
23	57	semi	Dip	3	12	1970 (91)	1993	1993
24	51	semi	Plant	4	1200	1975	1978	1979
25	42	retire	Spray/D	20	400	1965	1985	1985

Occu. now = whether participant is working or retired at time of assessment. Semi = semi –retired and the participant has reduced work activities significantly. Occu. then = occupation of participant at time of exposure. Dip/sup = government official supervising sheep dipping. Dip/trad = sheep trader. Dip/con = sheep dipping contractor. Dip/obs = observed dipping only. Spray/D = crop spraying in addition to sheep dipping, where the former made up most of the job. Plant = plant technician. Exposure index = the number of days per year spent using multiplied by the number of years spent using OPs. The years an individual gives for starting and stopping work do not necessarily tally with the number of years of exposure to OPs. The number in brackets represents the date where exposure to OPs commenced.

Exposure history

A summary of participants' exposure history is shown in Table 12. Most of the participants in this study had been exposed to OPs over a number of years as a result of sheep dipping, though four of these people were also involved in crop spraying. Two participants were only exposed to OPs through crop spraying and one individual was exposed to OPs through the course of his work as a plant technician.

Participants were asked to report, retrospectively on their exposure history. Unsurprisingly, some individuals had difficulty giving **precise** details about the duration and frequency of exposure (estimates were given instead) or the names of the chemical products used. Others were able to give detailed information and brought work diaries and receipts to interview. Participants used a variety of OP products of differing compositions, some individuals using more than one product during their lifetime.

Six individuals began farming in the 1950s and 1960s and are likely to have used organochlorine products before 1970, yet only two of them could remember the exact products used and the date when they changed to OPs[3]. However, in all cases the onset of ill health showed a close temporal relationship with the use of OPs and in all bar one case, the onset of ill health was in the 1980s and 1990s.

Quantifying exposure

Exposure history varied considerably despite participants appearing to have similar jobs (please see Table 13). The mean number of years spent working with OPs was 14 years (range 3-32, median 14) and the mean number of days per year spent working with OP products was 39 days (range 2-300 days, median 12). The lifetime exposure index varied tremendously with some individuals having as few as 12 days exposure to OPs throughout their lifetime and others having more than 3000 days exposure (mean 503 days, median 210, mode 400).

[3] OP dips were available in the 1960s but organochlorine based products were more commonly used. It was not until the 1980s that OPs began to dominate the market.

Table 13: Quantifying Exposure

	Mean	Range	Median
No. Years spent working with Ops	14 years	3-32	14
No days per year spent working with Ops	39 days	2-300	12
Lifetime Exposure Index (days)	503 days	12-3000	210

Protective clothing

All individuals used a minimal amount of protective clothing whilst working, such as wellington boots, boiler suits and waterproof leggings. Many sheep dippers stripped down to open shirts during the summer months and crop sprayers drove tractors with open cabs. Sixteen individuals were responsible for handling/mixing concentrate and they reported significant skin contamination following spillages, despite wearing rubber gloves. Furthermore, OP formulations based on organic solvents are liable to penetrate protective clothing unless contamination is washed off immediately. However, this is seldom possible. Sheep dip baths and crop spraying equipment required replenishing several times during the course of the day so agricultural workers were handling concentrate on more than one occasion during a working day. Twenty-two individuals reported cleaning out sheep dip baths or crop spraying equipment after use, resulting in further contamination. Sheep dippers were splashed with sheep dip during the course of their work and crop sprayers reported driving through mists of pesticide spray during their working day. In addition, sixteen individuals handled large numbers of sheep which had recently been dipped either at market or whilst shearing or rolling fleeces.

'Dippers Flu'

All participants reported that throughout their working life they suffered repeated episodes of flu-like symptoms (e.g. fatigue, muscle pain, headaches and general malaise) following exposure to OPs. The farming community refers to this phenomenon as 'dippers flu'. The cause and nature of 'dippers flu' has not been established scientifically, but the symptoms have much in common with those associated with mild exposure to organophosphate compounds and appear to share a temporal relationship with exposure to sheep dip. Few participants could date the first onset of flu-like

symptoms or estimate exactly how many episodes of flu-like symptoms they had suffered during their working life, but all could date the onset of more serious health problems. All participants reported that initially, the symptoms of 'dippers flu' lasted 24-48 hours and participants usually attributed them to the physical exertion involved in their work or the flu virus. However, over time participants found that it took longer and longer to recover from these episodes until they reached some sort of threshold[4] after which their symptoms persisted. Around this time, additional problems such as memory impairment and mood swings developed and several individuals stated that any re-exposure to OPs (or several other chemicals such as perfume, furniture polish, alcohol etc) exacerbated their condition. It is usually at this point that medical attention is sought for the first time.

Physical and psychological symptoms reported at interview

Table 14 shows the physical and psychological problems reported at interview and the percentage of participants who suffer from each symptom. Almost all participants complain of impaired memory and concentration and extreme fatigue. Aching joints, aching muscles, headaches, sleep disturbance and irritability are also frequently reported. Participants describe their symptoms as being severe and disabling and seventeen have felt unable to continue working and have retired on ill health grounds. The remainder of the cohort have significantly reduced their working activities and require the assistance of others (e.g. relatives or contract farm workers) to cope with their duties.

[4] Once an individual has reached their critical exposure threshold there is often a dramatic change in their health, for example several individuals collapsed or became disorientated and had an accident, others became bed-ridden for several weeks.

Table 14: Physical and psychological symptoms

Self-reported symptoms	Percentage of subjects reporting each symptom
Impaired memory and concentration	100%
Fatigue	92%
Aching joints and muscles	72%
Headaches	60%
Insomnia or hypersomnia	60%
Irritability and temper control problems	60%
Language difficulties	48%
Depression/low mood	48%
Chemical and alcohol intolerance	48%
Poor temperature regulation/sweating	43%
Gastro-intestinal problems	36%
Muscle weakness	32%
Chest/lung problems (pain, tightness, difficulty breathing)	28%
Anxiety	28%
Muscle twitching	28%
Loss of sensation in limbs	20%
Feeling slowed down (cognitively)	12%
Visual disturbances (e.g. blurred)	12%
Pins and needles in limbs	12%
Dizziness	12%
Urinary problems (e.g. increased micturition)	12%
Skin complaints	8%

Medical and Psychiatric Examinations

Over the years most of the participants have been examined by a range of medical professionals (neurologists, general physicians, cardiologists, neurophysiologists, psychiatrists) because of the severe and disabling nature of their symptoms. A number of individuals have collapsed (due to loss of consciousness or severe muscle weakness) and/or been involved in accidents as a result of cognitive impairment. On several occasions abnormalities have been detected on EEG, brain scan, neuropsychological or neurophysiological measures (please see Table 15). However, medical records frequently refer to such abnormalities as being of 'unknown significance' and the conclusion has often been drawn (according to medical notes) that the participant must be suffering from stress.

Table 15: Abnormalities detected during medical examinations.

Number tested	Medical test used	Number with abnormal results	Nature of findings
17	EMG	8 (47%)	Peripheral sensory and motor distal axonopathy.
6	Brain EEG	2 (34%)	Fronto-temporal slowing
10	Brain scans	3 (30%)	Frontal lobe damage (MRI); bilateral hypoperfusion (SPECT); cerebellum and brain stem atrophy (MRI; animal experiments have shown damage in these hindbrain structures following OP exposure, Abou Donia 2005)
12	Psychiatric assessment for medico-legal purposes.	Possibly 2 (17%)	The psychiatrists who assessed these individuals for medico-legal purposes concluded that in 10 cases there was no evidence of previous psychiatric history and the possibility that participants' symptoms could be related to exposure to OPs could be neither proved nor disproved. The possibility that the 2 remaining cases might have dementia or hysteria was raised.
14	Blood analysis Thyroid function	All normal	
14	Liver function	2 (14%)	Raised Gamma GT
14	Immune Function	12 (86%) Only one patient showed evidence of infection	Cytoxic suppressor cells reduced; TNF and interluken results indicate inflammation; low NK, Tand B cells; C-reactive protein raised;
11-13	Autonomic Function	8-11 (67-85%)	Low cardiac vagal tone, baroreflex cardioaccelerator failure consistent with exposure to pesticides
14	Bone biopsy	8 (57%)	Reduced bone mass compared to age matched peers.
15	Autoantibodies against neuronal cytoskeletal proteins	5 (34%)	Increased antibodies against neuronal cytoskeletal proteins in some patient sera is consistent with neurologic disorders resulting from exposure to pesticides.

EEG and brain scan data was collected independently from the medico-legal process and obtained from medical records. The remaining data was collected as part of the medical pilot study.

The author is not qualified to comment on the reliability, validity or clinical meaningfulness of some of the medical data provided in this table.

Summary

Detailed information about exposure history was obtained during a clinical interview and was found to vary considerably despite individuals having similar jobs. Participants used minimal protective clothing whilst working and all report episodes of dippers flu. Over time they took longer and longer to recover from these episodes until they reached a threshold after which symptoms persist. Participants report a range of ongoing physical and psychological problems (mainly fatigue, impaired memory, headaches, aching joints, insomnia and irritability) which are severe and disabling. Seventeen individuals have felt unable to continue working and the remainder have significantly reduced their working activities.

Evaluation of Hypothesis 1

Farmers are usually classified as having low-level exposure to OPs, but the findings from this cohort regarding multiple episodes of dippers flu, suggest this may not always be the case and that it may be more appropriate to classify some agricultural workers as having a history of undiagnosed acute poisoning. It may be misleading to restrict the definition of acute OP poisoning to episodes requiring medical intervention, since many individuals do not seek medical treatment for their symptoms, but retire to bed for a few days. It would be useful to develop diagnostic criteria of acute OP poisoning (similar to that seen in DSM-IV) based on specific symptoms and their temporal relationship to exposure.

SECTION 2: The nature and extent of cognitive impairment and mood disorder following exposure to OPs (quantitative data)

In order to investigate whether there were significant differences in mean scores on psychometric tests between the exposed and control cohorts, the data was analysed using unrelated t tests or the non-parametric equivalent (Mann Whitney U). Unless otherwise stated, all statistical tests were two-tailed. Where a predicted difference in performance was tested using more than one psychometric test, Larzelere and Mulaik tests were applied to control for Type 1 errors (Howell, 1992). This involved deriving revised alpha levels by using the formula depicted below:

$$\alpha^{\text{revised}} = \alpha^{\text{original}} / (k\text{-}i\text{+}1)$$

where α^{original}=.05; k= number of psychometric tests used; i= the rank order/strength of the original p value (rank 1 is assigned to the most significant findings).

Intelligence

Participants' performance on IQ tests is summarised in Table 16. The mean WAIS-R full scale and verbal IQs for the two groups were not significantly different from each other and were in the average range for the general population. A more detailed analysis of performance across the different subtests which make up the WAIS-R revealed that the exposed and control groups obtained significantly different results on three subtests: information, digit span and digit symbol, the exposed group performing more poorly than controls in each case. These findings remained unchanged even after controlling for Type 1 errors. The overall impression is that general intellectual ability is relatively well preserved in the exposed group, but there is evidence of patchy underfunctioning on tests of general knowledge, auditory verbal memory span (digit span) and psychomotor speed (digit symbol).

Table 16a: Overall Intelligence Test Scores

Test	Mean score	Standard deviation	t or U values	p value
WAIS-R Full IQ Patients n=25 Controls n=22	 102 109	 13.7 12.3	t=1.62	p=0.11
WAIS-R Verbal IQ Patients n=25 Controls n=22	 103 108	 13.9 12.6	t=1.31	p=0.19
WAIS-R Performance IQ Patients n=25 Controls n=22	 101 107	 12.4 9.6	t=1.99	p=0.05*

Table 16b: Intelligence Sub-Test Scores

Test	Mean score	Standard deviation	t or U values	p value	Revised α
Digit Symbol Patients n=25 Controls n=22	 7.8 10.4	 2.0 2.1	t=4.34	p=0.001*	0.005
Digit Span Patients n=25 Controls n=22	 8.5 10.9	 2.5 2.5	t=3.28	p=0.002*	0.005
Subtest age-scaled scores Information Patients n=25 Controls n=22	 10.2 12.3	 2.9 1.8	t=3.09	p=0.004*	0.006
Vocabulary Patients n=25 Controls n=22	 11.0 12	 2.4 2.1	t=1.76	p=0.08	0.006
Picture Arrangement Patients n=25 Controls n=22	 10.3 11.3	 2.4 2.0	t=1.61	p=0.12	0.007
Similarities Patients n=25 Controls n=22	 11.6 12.1	 3.2 2.3	t=0.70	p=0.49	0.008
Block Design Patients n=25 Controls n=22	 11.2 11.9	 2.6 2.7	U=255	p=0.67	0.01
Comprehension Patients n=25 Controls n=22	 11.5 11.8	 3.2 2.6	t=0.35	p=0.73	0.01
Picture Completion Patients n=25 Controls n=22	 11.3 11.1	 2.9 1.9	t=0.25	p=0.80	0.02
Objects Assembly Patients n=25 Controls n=22	 10.8 10.9	 2.3 1.7	t=0.25	p=0.80	0.03
Arithmetic Patients n=25 Controls n=22	 10.5 10.6	 2.6 2.5	t=0.15	p=0.87	0.05

Memory

In this section participants' performance on memory tests is summarised (see Table 17). Unrelated t tests (or Mann Whitney U) were used to investigate whether there were significant differences in mean scores on memory tests between the two groups.

Table 17: Memory Test Scores

Test	Mean score	Standard deviation	t or U values	p value	Revised α
Story recall delayed Patients n=25 Controls n=22	 14.7 36.8	 10.7 11.3	t=6.89	p<0.001*	0.005
List learning Recognition Patients n=14 Controls n=22	 7.4 13.3	 3.1 1.9	t=6.48	p<0.001*	0.005
List Learning 1-5 Patients n=14 Controls n=22	 32.5 49.1	 7.6 7.6	t=6.42	p<0.001*	0.006
Story recall immediate Patients n=25 Controls n=22	 20 34.5	 10.8 12.1	t=5.80	p<0.001*	0.006
List Learning Recall Patients n=14 Controls n=22	 5.4 10.5	 2.3 2.9	t=5.48	p<0.001*	0.007
Story recall retained Patients n=25 Controls n=22	 67.6 94.2	 26.6 13.5	t=4.41	p<0.001*	0.008
Complex figure delayed Patients n=14 Controls n=22	 57.7 83.9	 22.9 16.1	U=56	p<0.001*	0.01
Complex Figure immediate Patients n=14 Controls n=22	 63.9 86.7	 28.2 14.9	U=60	p=0.002*	0.01
Design Learning 1-5 Patients n=25 Controls n=22	 29.8 35.6	 9.3 6.4	t=2.46	p=0.018*	0.02
Complex figure retained Patients n=14 Controls n=22	 92 96	 19.2 10.4	U=98	p=0.07	0.03
Design learning recall Patients n=25 Controls n=22	 6.6 7.5	 2.6 1.7	t=1.35	p=0.19	0.05

Statistical analysis revealed that the groups differed significantly on all but two memory test scores even after controlling for Type 1 errors. The overall impression is that the exposed group is showing evidence of significant memory impairment.

Information Processing Speed

Potential differences between the two groups' performance on information processing tasks were investigated using Mann Whitney and t- tests. The results are summarised in Table 18.

Table 18: Information Processing Speed Scores

Test	Mean score	Standard deviation	U values	p value	Revised α
AMIPB Speed Patients n=25 Controls n=22	 32.3 52.1	 14.8 9.4	U=52	p<0.001*	0.01
Trail Making B Patients n=25 Controls n=22	 134.3 65.9	 68.7 20.1	U=58	p<0.001*	0.01
Trail Making A Patients n=25 Controls n=22	 60.4 30.6	 36.2 6.5	U=60.5	p<0.001*	0.02
AMIPB Task A Patients n=25 Controls n=22	 45.8 64.1	 18.8 14.0	U=120.5	p=0.001*	0.03
AMIPB Adjusted Patients n=25 Controls n=22	 56.8 71.7	 20.1 17.1	U=156	p=0.01*	0.05

Analysis of the groups' information processing speed scores revealed that the exposure group performed significantly slower/worse than the controls on all of the tests in Table 18 (even after controlling for Type 1 errors). Therefore, the overall impression is that there is evidence of impaired psychomotor speed in this cohort.

Language Skills

Here, participants' performance on language tests is summarised (see Table 19). Unrelated t tests (or Mann Whitney U) were used to investigate whether there were significant differences in mean scores on language tests between the exposed and control groups. Analysis of the groups' performance on language tests revealed that the exposure group obtained lower scores than the controls on fluency, naming and reading tests (even after controlling for Type 1 errors). The overall impression is that there is evidence of impaired language ability in this cohort.

Table 19: Language Skills Test Scores

Test	Mean score	Standard deviation	t or U values	p value	Revised α
NART Patients n=25 Controls n=22	 96 111	 13.4 6.7	t=5.02	p<0.001*	0.01
Verbal fluency Patients n=25 Controls n=22	 26.8 44.1	 13.6 11	t=4.73	p<0.001*	0.02
Semantic fluency Patients n=23 Controls n=22	 16 21	 5.6 4.6	t=3.13	p<0.01*	0.03
Naming Patients n=25 Controls n=22	 11.6 12.5	 1.9 0.8	U=184	p=0.05*	0.05

Visuo-Spatial Skills

Participants' performance on visuo-spatial tests is summarised in Table 20. Analysis of the groups' performance on visuo-spatial tasks revealed that the exposure group obtained lower scores on these tests than control subjects (even after controlling for Type 1 errors).

Table 20: Visuo-Spatial Skills Test Scores

Test	Mean score	Standard deviation	t values	p value	Revised α
Face Recognition Patients n=25 Controls n=22	 43.3 50.5	 4.2 4.1	t=5. 89	p<0.001*	0.03
Line Orientation Patients n=25 Controls n=22	 25.4 27.7	 3.8 2.7	t=2.34	p<0.05*	0.05

Frontal 'Executive' Function

Reasoning Tests (similarities and picture arrangement)

Performance on reasoning tests was in the average range (please see Table 16).

Verbal Fluency

Verbal fluency is sensitive to frontal executive dysfunction. As was mentioned previously, there was a significant difference in performance between the exposed and control group with the former performing more poorly.

Stroop Test

The Stroop test is a measure of mental flexibility, in particular, the ability to switch between competing response modes. Eighty eight percent of the exposed group failed this test, performing below the cut off for abnormality, compared to five percent of the control group. It was therefore easier to nominally classify these scores as either "pass" or "fail" for analysis. A chi-square test revealed a significant difference between the two groups' performance on the Stroop Test ($\chi^2 = 31.66$, $df = 1$, $p<0.001$), with the exposure group performing significantly worse than the controls.

Summary

The overall impression is that abstract reasoning ability is relatively well preserved in the exposed cohort, but they show evidence of reduced verbal fluency and mental flexibility.

Suboptimal Effort

Four participants failed Rey's malingering test. Low scores raise the possibility of incomplete effort, exaggeration or simulation of symptoms, but caution is advised in interpreting the results as evidence of malingering when patients show significant levels of cognitive impairment (see Chapter Four). All of the participants who failed Rey's test obtained NART scores in the low average range (IQ less than 84) and three individuals obtained WAIS-R full scale IQ scores in the low average range (IQ less than 86) suggesting low average premorbid ability or acquired cognitive impairment. Therefore, Rey's test may have been unsuitable for use with these individuals. Participants' performance on psychometric tests was re-analysed after removing the data from the four individuals who failed Rey's test. This did not alter the pattern of results significantly. Differences between the exposed and control groups remained significant with a few exceptions: WAIS-R performance IQ, naming, immediate recall of visual information on one memory test (AMIPB design learning 1-5) and line orientation. It is possible that the four individuals who failed the Rey test did so because they have visuo-spatial deficits.

Table 21: Group differences following removal of data from four individuals who failed Rey's 15 item test.

Tests	Data from all participants	Data from four individuals who failed Rey's test removed
WAIS-R Performance IQ	*	ns
WAIS-R information subtest	**	*
WAIS-R digit span subtest	**	**
WAIS-R digit symbol subtest	***	**
List learning 1-5	***	***
List learning recall 6	***	***
List learning recognition trial	***	***
Story recall: Immediate	***	***
Story recall: delayed	***	***
Story recall: retained	***	***
Design learning 1-5	*	ns
Complex figure: immediate	**	*
Complex figure: delayed	***	**
Trails A	***	**
Trails B	***	***
AMIPB: Task A	***	***
AMIPB:Speed	***	***
AMIPB:Adj	**	**
Verbal fluency	***	***
Semantic fluency	**	*
NART	***	**
Naming	*	ns
Face Recognition	***	***
Line Orientation	*	ns

* $p<0.05$
** $p<0.01$
*** $p<0.001$

Since the Rey 15 item test can be relatively insensitive to suspected malingering (Kelly, Baker, van den Broek, Jackson, Humphries, 2005) and may misclassify persons with

moderate to severe cognitive impairment (Lezak, 2004) the consistency of participants' performance was examined in two other ways.

All subjects completed Ravens Progressive Matrices which is composed of five problem sets which increase in difficulty. Raven (1996) suggests that if a person's score on one of these sets deviates by more than 2 points from their expected score (given the total score they attain on the matrices) it suggests inconsistent performance. This method identified five farmers, only one of whom had been identified by the previous method (i.e. Rey 15 item test) and four controls as showing inconsistent performance on this test. However, Raven's method is prone to both false positive and false negative errors. One study found false positive results were particularly high among low IQ and neuropsychologically impaired individuals (Gudjonsson & Shackleton, 1986).

Another method of analyzing performance on Ravens Matrices, with a more modest false positive rate of 5% (McKinzey, Podd, Krehbeil & Raven, 1999) is that reported by Gudjonsson & Shackleton in 1986. This involves the use of a statistical formula to measure the rate of decay (RDS) across Raven's five sets. The underlying assumption is that fakers show a smaller rate of decay because they fake disproportionately more of the easy items in the first two sets. When this method is applied to the subjects who took part in the present study, one farmer and two control subjects (previously identified by Raven's method) were identified as showing suboptimal effort. Re-analysis of the data after removal of this additional individual did not change the overall results of this study.

A final method of assessing symptom validity which was considered by the author given the data available in this study, but subsequently deemed inappropriate, was one which involves subtracting WAIS-R Digit Span subtest scores from WAIS-R Vocabulary subtest scores. The assumption underlying this method is that immediate attention span appears preserved relative to overall cognitive level in concussed patients. Thus, disproportionate reductions in digit span may be indicative of suboptimal effort in patients who have sustained mild head injury (Mittenberg, Theroux-Fichera, Zielinski,

Heilbronner, 1995). However, Millis, Ross & Ricker (1998) suggest that this method may not be an appropriate measure of effort in clinical groups other than traumatic brain injury, since digit span scores have been shown to be impaired in certain neurological conditions such as dementia (Lezak, 2004).

Indeed this method is unlikely to be a suitable measure of suboptimal effort in patients who have been exposed to neurotoxins as Digit Span, along with various other subtests from the WAIS-R has proven sensitive to subclinical toxic exposure effects. Indeed the World Health Organisation and the National Institute of Occupational Safety & Health have advocated a core battery, of six tests selected for their known sensitivity to neurotoxicity effects, and this battery includes Digit Span (Hartman, 1988).

Table 22: Performance on three measures of effort

Subject ID	Rey 15 item test	Raven: Consistency	Raven: RDS
Exposed Cohort			
1	X		
4		X	X
7	X		
10	X	X	
11	X		
17		X	
18		X	
19		X	
Control Cohort			
29		X	
31		X	
42		X	X
45		X	X

Mood State

The 'Hospital Anxiety and Depression Scale' was administered to all individuals. It is a 14 item self-assessment questionnaire designed to screen for clinically significant levels of anxiety and depression in individuals who attend hospital departments. The items are based solely on the psychic manifestations of anxiety and depression. Symptoms of somatic reference are excluded so that scores are not affected by the physical health of the respondent.

Seventy two percent of the exposed cohort were found to be suffering from clinically significant levels of anxiety. Twelve percent were suffering from mild levels of anxiety, thirty six percent from moderate levels and twenty four percent from severe levels of anxiety. Only 23% of control subjects were suffering from anxiety and in all cases levels were mild.

Seventy six percent of the exposed cohort were found to be suffering from clinically significant levels of depression. Sixteen percent were suffering from mild levels, thirty six percent from moderate levels and twenty four percent from severe levels of depression. None of the control subjects were clinically depressed.

Table 23: Percentage of individuals suffering from clinically significant levels of anxiety and depression (controls in parenthesis).

Mood State	Mild	Moderate	Severe	Total
Anxiety	12% (23%)	36% (0%)	24% (0%)	72% (23%)
Depression	16% (0%)	36% (0%)	24% (0%)	76% (0%)

Controlling for the effects of depression

Previous research has shown that depression may be related to poor performance on certain psychometric tests, particularly those relating to memory (Watts, 1996). It is therefore standard practice to take account of participants' depression scores when analysing their performance on neuropsychological tests. Table 23 shows the mean depression scores for the exposed and control groups and the difference between them.

Table 24: Group performance on depression scale

	Mean score	Standard deviation	U value	p value
Patients n=25 Controls n=22	10.9 2.0	4.4 1.6	19.00	p<0.0001

Analysis of covariance (ANCOVA) was used to assess group differences on psychometric tests after the potentially confounding effects of depression were statistically removed. Bootstrap ANCOVAs were used for abnormally distributed variables (naming, Trails A and B, Stroop, Rey copy). The results are summarised in Table 24.

Table 25: Group differences after the potentially confounding effects of depression were statistically removed

Tests	Not accounting for depression	Accounting for depression
NART IQ	***	
WAIS-R Performance IQ	*	
WAIS-R Information subtest	**	
WAIS-R Digit Span subtest	**	
WAIS-R Digit Symbol subtest	***	
Verbal Fluency	***	*
Semantic Fluency	**	
Naming	*	
List Learning Trials1to5	***	*
List Learning Trial 6	***	*
List Learning Trial B	***	
List Learning -Recognition	***	**
Design Learning Trials1to5	*	
List Learning Trial B	***	
Story Recall - Immediate	***	*
Story Recall - Delayed	***	**
Story Recall - Retention	***	**
Trails A	***	
Trails B	***	*
AMIPB – IP Speed	***	
AMIPB – Motor Speed	***	
AMIPB – Mental Speed	**	
Stroop	***	***
Line Orientation	*	
Face Recognition	***	***
Figure Recall - Copy	***	***
Figure Recall - Immediate	**	
Figure Recall - Delayed	***	

*	sig <0.05
**	sig <0.01
***	sig <0.001

Significant differences between the groups on verbal memory tests and tests of mental flexibility (i.e. Stroop and Trails B) remain, despite partialling out the potentially confounding effects of depression. Significant differences between the groups on tests of

visual memory, psychomotor speed and auditory working memory span were lost after controlling for depression. It is not surprising that some of these effects were lost given the strong correlation between depression and group (Rho=-0.81). This indicates that the controls and the exposed participants could almost be differentiated from one another by their depression scores alone. Thus trying to account for the effect of depression on the participants' scores would almost be like attempting to factor out the effect of the group to which they belonged. This would inevitably knock out many significant differences between the two groups' test scores.

Another way of looking at the effect of depression on test scores is to compare the performance of neurological and psychiatric patients with the exposed cohort from this study. Coughlan and Hollows (1984; 1985) provide data on the sensitivity of the AMIPB subtests to depression and cerebral dysfunction by reporting the incidence of poor performance amongst neurological patients and a group of depressed psychiatric patients. The tables below show how the incidence of poor performance amongst the exposed cohort from this study compares to that seen in the subjects described by Coughlan and Hollows. This gives a general indication of how the groups' performance compares, but it is important to note that the groups are not well matched to the OP cohort on important variables such as age. For example, the vast majority (86%) of Coughlan and Hollows neurological patients were under the age of 45 years, whilst only 32% of the OP cohort were in this age band, 68% being between 46 and 65 years of age.

Chi square analysis (please see Tables 25-27) reveals that the exposed cohort in this study show a higher incidence of poor performance on several verbal memory tests than both neurological and psychiatric patients and this may indicate that a combination of organic and psychological factors account for the verbal memory impairment seen in this cohort. The incidence of impairment on visual memory tests amongst the OP cohort is lower than that seen in neurological patients and not significantly different from that seen in psychiatric patients. Similar rates of impairment on information-processing tasks are seen amongst the OP cohort and neurological patients.

Table 26: Differences in the percentage of people who performed at or below two standard deviations below the mean score on verbal memory tests between the OP cohort, neurological and psychiatric patients.

Verbal Memory Tests	OP cohort (n=20) Vs Neurological (n=54)		OP cohort (n=20) Vs Psychiatric/Depressed (n=48)	
	χ^2	p	χ^2	p
Story Recall Immediate	4.93	**		
Story Recall Delayed	1.96	-		
Story Recall % Retained	3.20	*	15.37	****
List Learning Trials 1-5	1.93	-	12.35	****
List Learning Trial 6	0.78	-		

- not significant,*p<0.10, ** p<0.05, ***p<0.01, ****p<0.001

Table 27: Differences in the percentage of people who performed at or below two standard deviations below the mean score on visual memory tests between the OP cohort, neurological and psychiatric patients.

Visual Memory Tests	OP cohort (n=20) Vs Neurological (n=54)		OP cohort (n=20) Vs Psychiatric/Depressed (n=48)	
	χ^2	p	χ^2	p
Complex figure immediate	2.73	*		
Complex figure delayed	1.40	-		
Complex Figure retained	2.40	-	1.02	-
Design learning 1-5 (rote)	3.05	*	0.09	-
Design learning 6 (delayed)	3.62	*		

- not significant,*p<0.10, ** p<0.05, ***p<0.01, ****p<0.001

Table 28: Differences in the percentage of people who performed at or below two standard deviations below the mean score on AMIPB Information Processing Tests between the OP cohort, neurological and psychiatric patients.

AMIP Information Processing Tests	OP cohort (n=25) Vs Neurological (n=54)	
	χ^2	p
Psychomotor speed (Task A)	0.003	-
Motor Speed	1.05	-
Mental Speed	1.54	-

- not significant,*p<0.10, ** p<0.05, ***p<0.01, ****p<0.001

Summary of Results

Analysis of participants' performance on neuropsychological tests indicates that general intellectual ability is relatively well preserved in the OP cohort, but they show evidence of patchy underfunctioning on tests of auditory verbal memory span (digit span) and psychomotor speed (digit symbol), relative to healthy controls. Abstract reasoning ability appears relatively well preserved in the OP cohort, but they show evidence of reduced mental flexibility and verbal fluency. The OP cohort show evidence of impaired psychomotor speed relative to healthy controls and rates of impairment are similar to those reported in a group of neurological patients. The OP cohort perform significantly worse than healthy controls and an unmatched group of neurological patients, on memory tests, particularly verbal memory tests and their performance in this regard may have been affected by a combination of psychological and organic factors. Indeed, the majority of participants showed signs of mood disorder with 70% of the cohort found to be suffering from clinically significant levels of anxiety and 75% of the cohort were suffering from clinically significant levels of depression. Previous research has shown that depression may be related to poor performance on certain psychometric tests, particularly those relating to memory and so it is standard practice to take account of participants' depression scores when analysing their performance on neuropsychological tests. Significant differences in performance on tests of verbal memory and mental flexibility persist even after the effects of depression are removed. However, it is

important to note that the mood disorder seen in the OP cohort may reflect direct neurological damage following exposure to OP chemicals (please see Chapter Six for further discussion) and therefore it may be inappropriate to try and remove this factor from analyses.

Table 29: Cognitive and emotional deficits observed in this cohort.

High rates of impairment	Low rates of impairment	Preserved ability
Working memory span	Visual memory	General Intelligence
Verbal learning	Visuo-perceptual skills	Naming
Psychomotor speed		Abstract Reasoning
Verbal fluency		
Semantic fluency		
Mental flexibility (Stroop)		
Anxiety		
Depression		

Evaluation of hypotheses 1 and 3

Participants show a similar pattern of deficits to that seen in individuals with a history of chronic low-level exposure to OPs (see Chapter Three), in particular, they have high rates of emotional distress and perform poorly on tests of psychomotor speed, information-processing speed and attention. In addition, they show evidence of executive dysfunction, memory and language deficits and in this respect they have more in common with individuals who have a history of acute OP poisoning (please see Chapter Six for further discussion).

SECTION 3: Relationship between neurobehavioural problems and indices of exposure.

Pearson product moment correlations and linear regression were used to establish whether there is a relationship between cognitive function and exposure history. The OP cohort perform significantly worse than healthy controls on a range of psychometric tests. In this section analyses are undertaken to determine whether there is a linear relationship between indices of exposure and cognitive function within the exposed cohort. It was predicted that performance will worsen with increased exposure. Therefore, due to the unidirectional nature of the hypothesis, one tailed tests of significance were used.

Please see Table 29 for a summary of the results. Significant, negative correlations were observed between the lifetime exposure index and immediate and delayed recall of a story ($r = -.34$, $p<.05$; $r = -.40$, $p<.05$) indicating an association between prolonged and intense exposure and lower scores on a story recall test. However, the lifetime exposure index did not correlate with any of the other psychological variables.

Duration of exposure correlated with a number of variables. For example, significant, negative correlations were observed between duration of exposure and WAIS-R Verbal IQ ($r = -.43$, $p<.01$), working memory span/digit span ($r = -.34$, $p<.05$), immediate and delayed recall of a passage of prose ($r = -.34$, $p<.05$; $r = -.42$, $p<.01$) and motor speed ($r = -.35$, $p<.05$). This indicates that in this cohort, lower scores on tests of motor speed, verbal and memory functioning are associated with increased duration of exposure.

A significant positive correlation was observed between WAIS-R Verbal IQ scores and the time elapsed since the last exposure ($r =.47$, $p<.01$), indicating that those individuals whose last episode of exposure was in the more distant past obtained higher IQ scores. This could also mean that IQ scores might improve over time if exposure is avoided, though further research would be required to address this properly. Negative correlations

were also observed between the onset of ill health and delayed story recall scores ($r = -.38$, $p<.05$), verbal fluency ($r = -.35$, $p<05$) and mental information processing speed ($r = -.36$, $p<.05$), indicating that those individuals who have been ill for a prolonged period of time perform more poorly on these psychometric tests.

Negative correlations were also observed between severity of depression and WAIS-R Verbal IQ ($r = -.39$, $p<.05$) immediate and delayed story recall ($r = -.41$, $p<.05$; $r = -.35$, $p<.05$) and motor speed ($r = -.43$, $p<.01$). Higher scores on a depression questionnaire are associated with lower scores on WAIS-R verbal subtests, the ability to retain prose and motor speed. Severity of depression did not correlate significantly with any of the exposure indices.

Age did not correlate significantly with either duration of exposure ($r=.222$, $p=.144$) or the lifetime exposure index ($r=.076$, $p=.359$) so these exposure variables are unlikely to be an indicator of age.

Table 30: Pearson correlations between psychological variables and indices of exposure.

Test	Total Exposure Index	Duration Exposure	Last Exposure	Onset of ill health
WAIS-R Verbal IQ	-.09, ns	-.43*, p<01	.47*, p<01	.00, ns
Digit span	-.18, ns	-.34*, p<05	-.07, ns	-.24, ns
Digit symbol	.07, ns	.00, ns	-.07, ns	-.14, ns
List Learning Trials1-5	.02, ns	-.00, ns	-.12, ns	-.32, ns
List learning Trial 6	.03, ns	.11, ns	-.22, ns	-.12, ns
Story Recall Immediate	-.34*, p<.05	-.34*, p<.05	-.05, ns	-.28, ns
Story recall Delayed	-.40*, p<.05	-.42*, p<01	.01, ns	-.38*, p<05
Verbal Fluency	-.14, ns	-.30, ns	.15, ns	-.35*, p<05
Stroop	.09, ns	.19, ns	-.31, ns	-.12, ns
AMIPB Motor speed	-.19, ns	-.35*, p<05	.07, ns	-.23, ns
AMIPB Mental speed	-.27, ns	-.16, ns	-.25, ns	-.38*, p<05
Anxiety	.13, ns	.26, ns	-.17, ns	-.06, ns
Depression	.03, ns	.19, ns	-.17, ns	-.13, ns

ns = not significant

Linear Regression

Linear regression analyses were performed to determine whether the effects of exposure remain after controlling for the potentially confounding effects of age, premorbid IQ and depression and to establish the relative contribution of confounding and exposure variables to the variance in psychometric test scores.

Age, premorbid IQ and depression explain 29% of the variance in WAIS-R Verbal IQ scores (adjusted R square = .292; $F(3,21) = 4.30$, $p < .05$). When exposure variables were

added the overall variance accounted for increased by .13 and this increase just missed being significant at $\alpha < .05$ (Change in $R^2 = 13\%$; $F_{change}(2,19) = 2.51$, $p = .055$). Overall, the model with all variables included was significant ($F(5,19) = 3.96$, $p < .05$). Table 30 presents the unstandardized regression coefficients for the final model and related test statistics as well as the percentage of variance in the dependent variable uniquely accounted for by each variable in the analysis (partial r^2). As can be seen in the Table, premorbid IQ predicted WAIS-R VIQ independently of the other predictors in the analysis (accounting for 20% of the variance). One of the exposure variables (last exposure) almost reached significance.

Table 31: Regression coefficients illustrating the effect of exposure variables, demographic factors and premorbid IQ on WAIS-R Verbal IQ scores.

Model	B	t	significance	Partial r^2 expressed as %
Age	.079	.347	.73	0.6%
Depression	-.848	-1.619	.12	12%
Premorbid IQ	11.656	2.167	.043	20%
Duration of Exposure	-.195	-.647	.26	2%
Last Exposure	.888	1.639	.06	12%

Age, premorbid IQ and depression explain 1% of the variance in WAIS-R digit span scores (adjusted R square = .-.012; $F(3,21) = .904$, $p = .46$). When exposure variables were added the overall variance accounted for increased by .12 which was a significant increase at $\alpha < .05$ (Change in $R^2 = 12\%$; $F_{change}(1,20) = 3.23$, $p < .05$). Overall, the model with all variables included was not significant ($F(5,19) = 1.56$, $p = .22$). Table 31 presents the unstandardized regression coefficients for the final model and related test statistics as well as the percentage of variance in the dependent variable uniquely accounted for by each variable in the analysis (partial r^2). As can be seen in the Table,

only duration of exposure predicted WAIS-R digit span scores independently of the other predictors in the analysis (accounting for 14% of the variance).

Table 32: Regression coefficients illustrating the effect of exposure variables, demographic factors and premorbid IQ on WAIS-R digit span subtest scores.

Model	B	t	significance	Partial r^2 expressed as %
Age	-.027	-.558	.58	1.54%
Depression	-.044	-.398	.69	0.8%
Premorbid IQ	-1.625	-1.415	.09	9%
Duration of Exposure	-.104	-1.797	.04	14%

Age, premorbid IQ and depression explain 13% of the variance in immediate story recall scores (adjusted R square = .127; $F(3,21) = 2.165$, $p = .122$). When exposure variables were added the overall variance accounted for increased by .093 and this increase was not significant at $\alpha < .05$ (Change in $R^2 = 9\%$; $F_{change}(2,19) = 1.31$, $p = .15$). Overall, the model with all variables included was not significant ($F(5,19) = 1.86$, $p = .15$).

Age, premorbid IQ and depression explain 6% of the variance in delayed story recall scores (adjusted R square = .056; $F(3,21) = 1.473$, $p = .251$). When exposure variables were added the overall variance accounted for increased by .227 and this increase just missed being significant at $\alpha < .05$ (Change in $R^2 = 23\%$; $F_{change}(3,18) = 2.28$, $p = .06$). Overall, the model with all variables included was not significant ($F(6,18) = 2.01$, $p = .12$).

Table 33: Regression coefficients illustrating the effect of exposure variables, demographic factors and premorbid IQ on delayed story recall.

Model	B	t	significance	Partial r^2 expressed as %
Age	-.028	-.128	.90	0.09%
Depression	-.888	-1.901	.07	17%
Premorbid IQ	-4.018	2.167	.43	3%
Exposure Index	.050		.43	0.2%
Duration of Exposure	-.414	-.647	.09	9%
Onset of ill health	.667	1.639	.09	9%

Age, premorbid IQ and depression explain 11% of the variance in verbal fluency scores (adjusted R square = .108; $F(3,21) = 1.97$, $p = .15$). When exposure variables were added the overall variance accounted for increased by .067 and this increase was not significant at $\alpha < .05$ (Change in $R^2 = 7\%$; $F_{change}(1,20) = 1.89$, $p = .09$). Overall, the model with all variables included was not significant ($F(4,20) = 2.011$, $p = .13$).

Age, premorbid IQ and depression explain 52% of the variance in motor speed scores (adjusted R square = .516; $F(3,21) = 9.52$, $p < .05$). When exposure variables were added the overall variance accounted for increased by .003 and this increase was not significant at $\alpha < .05$ (Change in $R^2 = 0.3\%$; $F_{change}(1,20) = .130$, $p = .36$). Overall, the model with all variables included was significant ($F(4,20) = 6.88$, $p < .05$). Table 30 presents the unstandardized regression coefficients for the final model and related test statistics as well as the percentage of variance in the dependent variable uniquely accounted for by each variable in the analysis (partial r^2). As can be seen in the Table, premorbid IQ and depression predicted motor speed scores independently of the other predictors in the analysis (accounting for 62% of the variance).

Table 34: Regression coefficients illustrating the effect of exposure variables, demographic factors and premorbid IQ on motor speed scores.

Model	B	t	significance	Partial R^2 expressed as %
Age	-.029	-1.577	.130	11%
Depression	-.099	-2.385	.027	22%
Premorbid IQ	1.570	3.683	.001	40%
Duration of Exposure	-.008	-.360	.36	0.6%

Age, premorbid IQ and depression explain 3% of the variance in mental speed scores (adjusted R square = .027; $F(3,21) = 1.23$, $p = .33$). When exposure variables were added the overall variance accounted for increased by .08 and this increase was not significant at $\alpha < .05$ (Change in $R^2 = 8\%$; $F_{change}(1,20) = 2.15$, $p = .08$). Overall, the model with all variables included was not significant ($F(4,20) = 1.50$, $p = .24$).

Summary

Statistical analyses were carried out to look at the relationship between exposure history and cognitive and emotional deficits. Significant correlations were observed between a number of exposure variables and verbal IQ, working memory span (digit span), verbal fluency, story recall and information processing speed. However, when the potentially confounding effects of age, premorbid IQ and mood were controlled for, the association between exposure and verbal fluency, immediate story recall, mental and motor speed was removed. This indicates that the effect between exposure and performance on these cognitive tests was confounded by these background factors. The association between exposure and verbal IQ and delayed story recall just failed to reach significance; whilst the association between exposure and digit span remained significant.

CHAPTER SIX: DISCUSSION

Overview

The present study compared the neuropsychological performance of 25 agricultural workers with a history of exposure to organophosphate pesticides with 22 non-exposed controls (matched for age, gender, years in education and estimated premorbid IQ). A range of cognitive and emotional problems were identified in agricultural workers. Although general intellectual ability was relatively well preserved in the exposed cohort, they obtained lower scores on tests of auditory verbal memory span, verbal learning, verbal fluency, mental flexibility, reading, visuo-spatial skill and information processing speed, than non-exposed controls. In addition, over 70% of the exposed cohort complained of clinically significant levels of anxiety and depression. They also reported a range of physical symptoms, the most prominent being fatigue, aching muscles and joints, headaches, sleep disturbance and irritability. Agricultural workers describe their symptoms as being severe and disabling and two thirds of them have retired on ill health grounds. The remainder have reduced their working activities.

All of the exposed cohort attribute their problems to OPs poisoning and were pursuing personal injury claims against chemical manufacturers or employers. Surprisingly, exposure history varied enormously amongst individuals who appeared to have very similar jobs, a factor which has not been commented on by previous research. As a result, this was not a homogeneous group of individuals, despite appearing so on the surface and it was impossible to attribute specific symptoms to particular OP products, doses or routes of exposure. Agricultural workers were convinced that OPs were to blame for their difficulties, because of the close temporal relationship between exposure to OP formulations and the onset of symptoms. All participants used minimal protective clothing when working with these compounds because it impairs mobility, and as a result, significant skin contamination was reported. All of the participants complained of multiple episodes of flu-like symptoms, which commenced during exposure and lasted 24-48 hours afterwards. These symptoms have much in common with those associated

with mild to moderate OP poisoning. Maximum cholinesterase depression occurs 8-24 hours after exposure to OPs and symptoms take 4-8 hours to reach maximum severity. Cholinergic levels may take up to 3 months to stabilize (Mearns, Dunn and Lees-Haley, 1994). Farmers are usually classified as having low-level exposure to OPs, but dippers flu may reflect undiagnosed, mild toxicity and it may be more appropriate to classify some agricultural workers as having a history of acute poisoning. Over time agricultural workers found it took longer and longer to recover from these episodes of dippers flu until they reach some sort of threshold after which physical symptoms persist and cognitive and emotional symptoms develop. It is usually at this point that medical attention is sought. Twelve agricultural workers were found to have abnormal immune system function, of unknown significance; five had increased autoantibodies to cytoskeletal proteins which is indicative of axonal degeneration secondary to neuronal death and is consistent with chemical-induced nervous system injury (Abou-Donia 2005); eight had peripheral, sensory and motor distal axonapathy; and eleven had a pattern of autonomic nervous system dysfunction, consistent with that seen in other farmers who have been exposed to OP pesticides, whereby target organs in the skin, large blood vessels, heart and brain are affected (Julu, Hansen & Jamal, 2005).

Statistical analyses were carried out to look at the relationship between exposure history and cognitive and emotional deficits. A number of significant correlations were observed between exposure variables and verbal IQ, working memory span (digit span), verbal fluency, story recall and information processing speed. However, when the potentially confounding effects of age, premorbid IQ and mood were controlled for, some of these correlations became non significant. Others were only moderately altered whilst the association between exposure and working memory span remained significant. There are two potential explanations for these results (1) an association does not exist between exposure and cognitive function, or (2) this study does not have the power to reliably detect a relationship between exposure and cognitive function once the potentially confounding effects of other variables are removed, because the sample size is too small.

Although the association between exposure and performance on some cognitive tests overlapped with background factors, particularly premorbid IQ, evidence from group analyses suggests that premorbid IQ alone may not account for the differences in test results. Group analyses, where an unexposed control group was matched to the exposed group in terms of age and premorbid IQ, found differences between the groups in performance on psychometric tests. Thus, both correlation and group analyses suggest a relationship may exist between exposure and performance on specific neuropsychological tests.

Comparisons with previous research

Cognitive deficits

As an occupational group, agricultural workers are considered unlikely to suffer episodes of acute OP poisoning, so the present findings were compared, initially, with those of previous research concerning the neurobehavioural sequelae of long-term, low-level exposure to OPs. However, interpreting the results of studies of low-level exposure is complicated by the fact that many have included subjects with a history of acute poisoning, or have not provided sufficient information to convince the reader that participants do not have a history of acute poisoning (Bosma et al, 2000; Rodnitzky et al, 1975; Korsak and Sato, 1977).

Five out of thirteen studies concerning the effects of long-term exposure to OPs, found no evidence of cognitive impairment in workers who do not have a history of acute poisoning requiring medical intervention. Three of these studies may have obtained negative results because of methodological weaknesses such as the selection of an inappropriate control group (Rodnitzky et al, 1975); use of psychometric tests which lack sensitivity (Albers et al, 2004); division of study participants into groups based on an assumption about a likely relationship between peripheral and central nervous system dysfunction (IOM study, 1999). The remaining studies examined pest control operators with a very short history of exposure to OPs and their results seem to indicate that if

episodes of acute poisoning can be avoided (including undiagnosed, mild toxicity), significant neurological sequelae can be prevented in the short term (Ames et al, 1995; Steenland et al, 2000). However it is important to note that exposed subjects in Steenland et al's study reported more symptoms suggestive of neurological impairment than controls, though few significant differences were found between the groups on quantitative tests. The remaining eight studies report a range of deficits in workers chronically exposed to OPs. All found evidence of reduced reaction time, psychomotor or information-processing speed, but inconsistent results were reported in relation to memory functioning, with some studies finding working, visual and verbal memory deficits (Bosma et al, 2000; Roldan-Tapia et al, 2005; Srivastava et al, 2000; Farahat et al, 2003) whilst others do not (Korsak & Sato, 1979; Maizlish et al 1987; Fiedler et al, 1997; Stephens et al, 1995). The study by Stephens et al (1995) is similar to the current study in that UK farmers who had been exposed to OPs in sheep dip were examined. Stephens et al (1995) found that in comparison to controls, farmers performed more poorly on tests of sustained attention syntactic reasoning and information-processing speed and had higher rates of emotional distress. Unlike the findings of this study, memory functioning was found to be intact. There could be two reasons for this discrepancy:

(1) Stephens et al (1995) used a limited test battery which may lack sensitivity, whereas the current study used tests which are known to be sensitive to the effects of organic brain damage and are routinely used in clinical practice.

(2) Sample differences - the participants in each study may differ in some important way, for example, participants in the current study may differ genetically and be more vulnerable to the effects of OPs; they may have used less protective clothing. All of the participants in Stephens et al (1995) study were fit enough to be in employment, whereas many of the participants in the current study had retired on ill health grounds. The participants in the current study have a history of 'dippers flu', but Stephens et al (1995) do not say whether their participants suffered from dippers

flu. In fact, Stephens et al provide very little information regarding their participants' exposure history making it difficult to compare results.

The pattern of deficits observed in this cohort has more in common with that seen following acute poisoning. Indeed, study participants may have suffered episodes of acute poisoning in that they all have a history of dippers flu, though at present, the possibility that dippers flu reflects acute poisoning is considered unproven by the scientific community (although a research project was commissioned recently by MAFF to explore this issue further). Previous studies concerning persistent effects following acute poisoning, have found evidence of attentional deficits, psychomotor slowing, greater vulnerability to psychiatric disorder, memory impairment, language deficits and executive dysfunction (Metcalf and Holmes, 1969; Savage et al, 1988; Kurlychek and Morrow 1989; Rosenstock et al, 1991; Reidy et al, 1992; Steenland et al, 1994; Misra et al 1994; Amr et al 1997; Stallones and Beseler 2002; Salvi et al 2003). Indeed, the results of this study are almost identical to the findings of Savage et al (1988) who examined 100 US workers with a history of acute poisoning (on average 9 years ago) and found that compared to matched controls, they obtained lower scores on tests of psychomotor speed, auditory working memory span, mental flexibility, vocabulary, reading and spelling plus they had higher rates of anxiety.

Of further interest is the similarity between the findings of the present study (i.e. working memory and learning deficits) and those of animal experiments. Prendergast, Terry and Buccafusco (1997, 1998) examined the effects of low-level exposure to organophosphates on memory functioning in rats and found that chronic exposure to OPs, insufficient to elicit symptoms of cholinesterase toxicity, impaired new learning in rats but not prior learning/knowledge. This impairment persisted even after withdrawal from OP exposure. AChE activity in the frontal cortex and hippocampus was suppressed (areas known to be involved in learning and memory) and hippocampal AChE activity recovered at a much slower rate than other brain regions. They conclude that extended exposure to OPs in industrial or agricultural settings may produce selective impairment

of working or short term memory, but may not significantly affect long term, reference memory.

Although many studies of workers exposed to OPs have found evidence of memory impairment following previous episodes of acute poisoning, different rates of verbal as opposed to visual memory have been found by different investigators (Kurlychek and Morrow, 1989; Savage et al, 1988; Rosenstock et al, 1991; Reidy et al, 1992; Misra, 1994; Steenland et al, 1994). This illustrates the importance of including psychometric tests which assess both verbal and visual modalities, yet some investigators have failed to do this (IOM study, 1999).

Emotional changes

Over 70% of the agricultural workers in this study were found to be suffering from significant levels of anxiety and depression and this is consistent with the findings of previous studies looking at the long-term effects of exposure to OPs (Gershon and Shaw, 1961; Rodnitzky et al, 1975; Levin et al, 1976; Amr et al, 1997; Stephens et al, 1995; IOM study, 1999; Ahmed and Davies, 1997, 1999 and 2000; Stallones and Beseler, 2002; Salvi et al, 2003; Roldan-Tapia et al, 2005).

The mood disorder seen in this cohort may be due to factors unrelated to exposure to OPs such as a stressful lifestyle or life events. Farmers are generally considered to lead a relatively stressful lifestyle and studies of stress in farming in the UK and other countries have consistently identified financial pressures, concern over farming policy and administration, time pressure, unpredictable weather conditions, pests and diseases, long hours of working and social isolation as important sources of stress in farmers (Simkin et al, 1998; Malmerg et al, 1999; McGregor, Willock and Deary, 1995).

However, in comparison to other occupational groups and the general population as a whole, the prevalence of mood disorder in farmers is relatively low. *A Survey of Psychiatric Morbidity* carried out in 2000 among adults living in private households in

Great Britain (Stansfeld, Head, Rasul et al, 2003), found that the prevalence of mood disorder to be much lower in 'farming related occupations' (around 2%), compared to the general population (13%) and the highest prevalence of 26% in sales occupations. Similar findings have been reported internationally. For example, Stallones and Beseler (2002) carried out an epidemiological study of farm residents in Colorado, USA, and found the prevalence of depression to be lower than the general population (6% vs 12.7%). Risk factors for depression amongst farm residents included being female, having income decline and poor health associated with exposure to pesticides.

In contrast to the low prevalence rates for mood disorder in farmers is the fact that they are one of the occupational groups in men who are at greatest risk of suicide (Charlton, Kelly, Dunnell et al, 1993). Malmerg et al (1997) suggest one reason for the discrepancy between the low prevalence of mood disorder and the high prevalence of suicide, may be factors other than occupational stress, such as the knowledge of how to kill themselves and availability of suicide methods (e.g. guns). It has been suggested that by having easy access to suicide methods farmers are at increased risk of carrying out and dying from an impulsive suicidal act (Malmerg et al, 1997; Booth, Briscoe and Powell, 2000; Thomas, Lewis, Thomas et al, 2003). Of further interest in this debate, is the fact that OPs can affect serotonin levels and low serotonin levels are associated with impulsivity and suicidal behaviour.

However, this study found little evidence to substantiate the view that the complaints seen in the current cohort might be secondary to psychological distress. Participants did not have a history of psychological problems nor did they report significant life-events or financial concerns which might be affecting their mood state. The mood disorder seen in this cohort may be secondary to direct neurological damage caused by exposure to OPs.

OPs are known to affect the central nervous system and changes in neurotransmitter function are known to influence mood. For example, insufficiencies in biogenic amines

(norepinephrine, epinephrine, dopamine, serotonin and histamine) are thought to underlie depression (the 'amine hypothesis' of depression). Antidepressants facilitate neural transmission in aminergic neurone systems whilst drugs which deplete these amines (e.g. Reserpine) may precipitate severe depression. OPs may affect mood as a result of their impact on the cholinergic system or they may influence other neurotransmitter systems such as serotonin, dopamine or glutamine (London, Flisher, Wesseling et al, 2005; Amr et al, 1997; Ahmed and Davies 1997). Animal experiments as long ago as the 1970s documented neurochemical inbalances in dopamine, serotonin and norepinephrine following exposure to OPs (Fatehyab-Ali, Hasan, Tariq, 1979). Ahmed, Davies and Freer have published a number of reports and articles (1995, 1997, 1999 and 2000) on this issue. In one paper (1997), they describe the clinical features (including cognitive impairment and emotional problems) of 33 agricultural workers with significant histories of exposure to OPs, seen through the course of routine clinical practice. Many of these patients suffered mood swings (irritability and depression) rather than pervasive depression and this instability was characteristic of organic mood disorder rather than functional affective disorder. Furthermore, these patients suffered excessive anticholinergic effects when given antidepressant drugs.

Only six of the exposed cohort in the current study were taking antidepressant medication (Prozac) at the time of assessment and another four had been prescribed low doses of Amitriptyline as an analgesic. A number of patients reported that they had been prescribed antidepressant medication in the past but found the side-effects difficult to tolerate. Ahmed and Davies conclude that long term exposure to OPs can cause mood disorder but they go on to state that although OPs are potent anticholinesterases, further research is needed into non-cholinergic toxic mechanisms of organophosphates, which might equally account for neurobehavioural symptoms (e.g. phosphorylation of glutamate or interference with second messenger systems).

Impact of mood on cognitive function

An important question is the extent to which cognitive function has been compromised by mood disorder as opposed to organic brain damage because the former may be amenable to treatment. However, the differentiation of functional and organic complaints is not always straightforward and depends upon the context and circumstances of presenting problems as well as clinical and neuropsychological features (Lezak, 2004).

Opinions differ as to the nature, extent and aetiology of cognitive impairment in anxious and depressed patients. This may reflect the different methodologies used by investigators, the populations studied and failure to control for potentially confounding variables such as medication and differences in intellectual ability between control subjects and study participants. Like CFS patients, depressed patients often perceive their memory functioning to be worse than it actually is with complaints about memory outstripping actual performance on psychometric tests. Although some investigators have found differences between control subjects and depressed patients on certain memory (prose recall, list learning, design learning, but working memory is usually intact) and information-processing speed tests (Watts, 1996; Zakanis, Leach and Kaplan, 1998), mood disorder is associated with a lesser degree of impairment than organic brain damage (Watts, 1996; Coughlan and Hollows, 1984). Differences in task performance between control subjects and those who are depressed are either non-existent or frequently small (less than one standard deviation) and this has led some researchers to suggest that poor performance may be secondary to lack of motivation. Indeed, depressed patients frequently show errors of omission or give 'don't know' responses, suggesting a lack of effort (Lezak, 2004). Impaired functioning is more common in patients with moderate to severe depression, in older patients (mean age >50 years) and those who take antidepressant medication (Austin, Mitchell, Wilhelm et al, 1999; Paradisio, Lamberty, Garvey et al, 1997; Zakzanis et al, 1998). Studies on younger, unmedicated patients (mean age 32-39 years) with mild to moderate levels of depression find few differences in task performance between control subjects and those who are

depressed (Grant, Thase and Sweeney, 2001; Porter, Gallagher, Thompson et al, 2003). The agricultural workers in the current study are equivalent to the younger, unmedicated subjects in these latter studies.

Trait anxiety seems to have little effect on performance but state anxiety has opposing effects on high and low ability subjects. Anxiety often enhances the performance of high ability subjects, but has a deleterious effect on low ability subjects. Working memory span is frequently affected by anxiety, whilst performance on other memory tests is often within normal limits, though subjects may have to expend more effort to succeed. Performance deteriorates when task demands are increased and if subjects experience failure (Watts, 1996).

In clinical practice, where relatively large test batteries are administered over a prolonged period of time, variation in the quality and consistency of performance may be observed in depressed and/or anxious patients. For example, inconsistencies are often observed between subjective reports and actual performance. Patients may show a pattern of global impairment due to variable performance between tasks and over time, as motivational levels fluctuate. Test scores may even fall below chance levels on occasion, if a patient becomes distracted/pre-occupied. Overall, the pattern of impairment seldom involves lateralised abilities or conforms to that seen following neurological damage (Lezak, 2004).

The findings of this study suggest mood disorder alone is unlikely to account for the cognitive deficits identified in agricultural workers, since the pattern of deficit differs from that seen in depression and anxiety in a number of ways: the severity of impairment greatly exceeds that seen in psychiatric patients and there was evidence of executive dysfunction in addition to memory and information-processing speed deficits. Participants did not show a pattern of global impairment and significant inconsistencies in performance between tasks and over time was not observed. Rather the pattern of deficits observed in this cohort suggests specific areas of dysfunction as opposed to

generalised dementia, which conforms to that seen following damage to subcortical brain structures which are known to be involved in mood regulation (Rogers, Bradshaw, Pantelis et al, 1998; please see 'Implications for Future Research', point (6)). Furthermore, significant differences between agricultural workers and control subjects on verbal memory tests and tests of mental flexibility remain despite partialling out the potentially confounding effects of depression on performance.

Physical symptoms

Agricultural workers in this study reported a range of physical and psychological symptoms during interview. Almost all complained of impaired memory and concentration and extreme fatigue. Aching joints, aching muscles, headaches, sleep disturbance and irritability were also frequently reported. The physical, cognitive and emotional symptom pattern seen in this cohort has common elements, but is diagnostically non-specific. The prevalence of these symptoms in the general population is high (Baxter et al, 2000), making it difficult to demonstrate unequivocally a causal link with OPs (Spurgeon, Gompertz and Harrington, 1997). Research has shown that individuals differ in their capacity to evaluate their own symptoms (Pitts and Phillips, 1991) and this means spontaneous self report is not an ideal method of evaluation since it can be influenced by a number of factors (e.g. selective recall, coping style, cultural factors, beliefs and attitudes). Well validated and psychometrically sound instruments are required to define and measure physical complaints objectively and to determine whether the symptom profile seen in agricultural workers who have been exposed to OPs differs from that seen in other populations.

The physical symptoms reported by agricultural workers in this study, are similar to those reported by other agricultural workers and occupational groups exposed to OPs. For example, the UK Veterinary Medicines Directorate (VMD) commissioned an analysis of 646 reports made to their Suspected Adverse Reaction Surveillance Scheme (SARSS) and a report was published in 2002. The following symptoms were frequently reported by agricultural workers following exposure to OPs: headache, dizziness,

parasthesia, fatigue, gastro-intestinal disturbance, depression, musculo-skeletal disorders, memory problems and respiratory disorders. In 2003 Tahmaz, Soutar and Cherie examined 63 respondents to the VMD SARSS scheme and found a high incidence of symptoms consistent with chronic fatigue syndrome in sheep farmers who use OP pesticides. Higher fatigue scores were associated with higher exposure to OPs. An epidemiological survey carried out in the UK of 367 sheep farmers who report ill health which they attribute to exposure to OPs found a high incidence of memory problems, headache, fatigue, aching muscles and joints, irritability, word-finding difficulties, depression, anxiety, sleep difficulties. This was even after excluding individuals with a medical history which might otherwise account for their symptoms. On average the health of those with a history of acute poisoning was worse than those without such a history (SHAPE study: Fletcher, MacLehose, Hurley et al, 2005).

Stallones and Beseler (2002) carried out an epidemiological survey of 761 farm residents in Colorado and found increased odds ratio for reporting neurological symptoms (memory problems, irritability, heart palpitations, headaches, sleep disturbance) in those with a history of acute poisoning. Salvi et al (2003) examined 37 Brazilian tobacco workers exposed to OPs and found they complained of fatigue, headache, hypertension and dermatitis. Ohayo-Mitoko et al (2000) compared 623 agricultural workers with 515 controls and found considerable changes in AChE inhibition during the spraying season (although levels were within normal limits) associated with symptoms of insomnia, anxiety, irritability and trembling. Srivastava et al (2000) compared 59 chemical plant manufacturers with 17 controls and found complaints of generalized weakness and fatigue were higher in the exposed cohort. Steenland et al (2000) compared 191 pest controllers with 189 controls and found the exposed group complained of neurobehavioural problems including memory problems, emotional states, fatigue and loss of muscle strength.

Finally, Julu et al (2005) suggest that fatigue may be secondary to autonomic nervous system dysfunction, particularly altered cardiac output. They examined 40 farmers who

developed chronic neurological symptoms following acute OP poisoning and found paradoxical resting bradycardia associated with low cardiac vagal tone, cardioaccelerator and baroreflex failure and abnormal cutaneous thermoregulation in more than 70% of farmers. Cardiac abnormalities of this nature make the heart slow to respond to sudden changes in physical activity and those affected experience this phenomenon as fatigue. OPs affect the cholinergic system, the major form of neurotransmission in the autonomic nervous system and the autonomic nervous system influences every major organ in the body. Julu et al suggest autonomic nervous system dysfunction may underlie many of the physical symptoms reported by farmers exposed to OP pesticides.

Differential Diagnosis

The possibility that the association between exposure to OPs and neurobehavioural problems occurred by chance in this study and is of no aetiological significance, must be considered along with the possibility that participants have made an attribution error.

Review of Medical records – Pre-existing medical complaints

The author reviewed participants' medical records to examine whether participants' symptoms might be attributable to other pre-existing medical conditions, but none of the study participants had anything of significance in their medical background, which might account for their ill health. However, it is possible that participants developed a new medical condition, such as chronic fatigue syndrome or mood disorder, which simply by chance, onset around the same time as they were exposed to OPs. Alternatively, their symptoms may be attributable to solvent poisoning, rather than OP poisoning or compensation neurosis.

Solvent poisoning

Chapter One, paragraph one explains that pesticide preparations include carrier substances (e.g. solvents) in addition to the active ingredient to improve absorption. These inert substances frequently comprise a large part of the commercial product and

may be neurotoxic in themselves. This is particularly true of solvents, though great differences exist in the neurotoxic potential of different solvents (Hartman, 1988).

As is the case with OP poisoning, the symptoms of solvent poisoning are nonspecific and can be mistaken for a host of other conditions such as emotional disturbance or influenza. The most prominent symptoms are fatigue, memory and concentration problems, emotional lability, depression, sleep disturbance, headaches, weakness, motor and sensory symptoms (Lezak, 2004). These symptoms are indistinguishable from those of OP poisoning, raising the possibility that solvents are in fact to blame for many of the cases of apparent OP poisoning.

There is no single test capable of diagnosing solvent intoxication and neurological examination is usually normal, except in severe cases (Hartman, 1988). In some cases, decreased neurofunctional capacity has been observed in cortical, particularly frontotemporal areas and subcortical structures, such as the thalamus, basal ganglia, hippocampus, amygdala, putamen (Morrow, Robin, Hodgson et al, 1992)

Neurobehavioural problems (mood disturbance, memory and attentional problems) are common and often seen early in an individual's exposure history. Neuropsychological studies have found evidence of reduced working memory span (digit span), psychomotor speed (digit symbol, Trails A & B, RT), mental flexibility, abstract reasoning, visuo-constructive ability (block design), memory impairment (visual memory seems to be affected to a greater degree than verbal memory) and visuo-perceptual deficits (Hartman, 1988; Lezak, 2004). The present study found evidence of reduced psychomotor speed, mental flexibility and working memory span in individuals who have been exposed to OP based pesticides, but abstract reasoning ability appeared intact and verbal memory was affected to a greater degree than visual memory, which is the opposite of what is usually seen following solvent intoxication.

Chronic fatigue syndrome (CFS)

Physical fatigue, memory and concentration problems were reported by all participants involved in this study and attributed to OP poisoning. However, these symptoms are fairly nonspecific and reported by many other groups of patients such as those suffering from depression or CFS.

Pawlikowska, Chalder, Hirsch et al (1994) carried out a population based survey of fatigue and psychological problems in 31651 individuals attending medical centres in the UK and found 18.3% of respondents reported substantial fatigue but the most common reason for this was lifestyle factors (work, family and social life). Only 1.4% of respondents described themselves as having chronic fatigue and myalgia and those who did were more likely to be female, of higher social class and to have a psychiatric history.

The term CFS is usually applied to people with a history of unexplained fatigue coupled with myalgia and other physical symptoms; but also includes people who are suffering from anxiety or depression; and those with medical conditions such as multiple sclerosis, which have been misdiagnosed (Report of the Royal Colleges of Physicians, Psychiatrists and General Practitioners, 1996).

As with OP and solvent intoxication, there is no specific laboratory abnormality associated with CFS. Indeed, CFS is not a disease with a recognised aetiology, but rather a clinical description of a constellation of symptoms which may have many different causes (e.g. viral infection, neuro-endocrine disorder, mood disorder, muscular abnormalities, and allergic reactions). The diagnosis of CFS is based on the clinical presentation and the exclusion of other disorders (Schmaling, DiClementi, Cullum et al, 1994). The hallmark of CFS is debilitating fatigue of at least 6 months duration accompanied by other symptoms such as fever, headache, painful lymph nodes, pharyngitis, myalgias, sleep disturbances, cognitive impairment and depression (Buchwald and Garrity, 1994). More recently operational criteria have been proposed by

three research groups, which differ in terms of the emphasis placed on somatic symptoms and the exclusion of psychiatric symptoms (Royal Colleges of Physicians, Psychiatrists and General Practitioners, 1996). None of these symptoms are specific to CFS and few are able to discriminate CFS from other disorders (Wessely and Powell, 1989).

Epidemiological studies regarding the prevalence of CFS have been complicated by the lack of agreement concerning the definition of CFS. The population point prevalence of CFS has been estimated to be around 2.6% in primary care (Royal Colleges of Physicians, Psychiatrists and General Practitioners, 1996) and the condition is more common in relatively young, well-educated women (Buchwald and Garrity, 1994). The functional impact of CFS can be profound and associated with impairment at work (Buchwald found that only 13-23% were in full-time employment), social and home environments. Various studies have confirmed that CFS is a chronic condition with a poor prognosis, for example Vercoulen, Swanink, Fennis et al (1996) examined the natural history of CFS over an 18 month period in 246 patients and found that only 3% recovered completely and 17% improved. Psychological factors were related to improvement (e.g. sense of control over symptoms and absence of physical attributions) rather than demographic factors or specialist treatment.

Psychological disorder is found in approximately 75% of CFS patients and can not be considered to be a reaction to disability, since rates are much higher than in other physical illnesses (Royal Colleges of Physicians, Psychiatrists and General Practitioners, 1996). Between 50-70% of CFS patients complain of considerable difficulties with memory and concentration and these difficulties are often implicated in occupational failure (McDonald, Cope and David, 1993). There have been at least 15 studies of cognitive function in CFS but the results of neuropsychological testing are inconsistent probably because of the heterogeneity of subject samples. To summarise the findings; studies have tended to find group means in the average to average range for general intellectual functioning. Visuo-spatial skills, reading ability and higher order skills such

as planning, abstract reasoning, verbal fluency and concept formation are generally unimpaired. Working memory span and composite scores on memory tests such as the Wechsler Memory Scale are within the normal range, but differences between CFS patients and healthy controls or patients with other medical conditions, may be apparent on particular subtests. The more effortful the task the more difficulty CFS patients have with acquisition of new material, but retention seems intact. This suggests their primary problem involves a reduction in speed of information-processing. Indeed, group differences are often observed on tests of reaction time, sustained attention, and psychomotor speed (CFS patients perform more poorly). However, scores are frequently within normal limits (Moss-Morris, Petrie, Large et al, 1996; Wearden and Appleby, 1996; Royal Colleges of Physicians, Psychiatrists and General Practitioners, 1996; Johnson, DeLuca and Natelson, 1996; Joyce, Blumenthal and Wessely, 1996; DeLuca, Johnson, Beldowicz et al, 1995; Schmaling et al, 1994; McDonald et al, 1993).

A consistent finding across studies is a discrepancy between objective performance and subjective reports of cognitive complaints in CFS patients and subjective reports are related to higher levels of psychopathology and somatic complaints. Several studies have found that CFS patients obtain similar scores to depressed patients on psychometric testing (Wearden and Appleby, 1996; DeLuca et al, 1995; Schmaling et al, 1994; McDonald et al, 1993; Wessely and Powell, 1989). It seems that CFS patients find the normal cognitive demands of everyday life subjectively fatiguing but perform normally on cognitive tests which are used to screen for organic brain disorder. In this respect they differ from the participants involved in the present study, many of whom obtained scores in the impaired range on tests of auditory verbal memory span, verbal learning, verbal fluency, mental flexibility and information-processing speed. In the present study, rates of impairment on verbal memory tests were higher than that seen in controls, psychiatric patients and neurological patients who had suffered mild cognitive impairment following generalised brain damage. Furthermore, the pattern of deficits seen in the OP cohort differs slightly from that seen in CFS patients in that working

memory span, retention of new material and verbal fluency were adversely affected in the OP cohort but this has not been observed in CFS patients.

Psychosomatic or somatoform disorder

The terms psychosomatic or somatoform disorder encompass several mental health disorders in which patients report physical symptoms which appear to be caused by or worsened by psychological factors. These symptoms cause significant distress and interfere with daily functioning. It is possible that the symptoms described by agricultural workers in this study have been initiated or maintained by psychological factors.

As many as 1 in 5 consultations in primary care are for somatic symptoms for which no specific cause can be found. Individuals with psychosomatic disorder make 2-3 times as many visits to physicians and generally complain of a large number of symptoms which recur but do not usually indicate major disease and for which they take many medications. Psychosomatic disorders are particularly common amongst out-patients and are more frequent in certain specialist clinics e.g. gastroenterology and cardiac clinics. These disorders are associated with a family history of illness, neurotic personality, early trauma, coping style, psychiatric disorder and secondary gain. They are more common in women, tend to occur between the ages of 18-30years, are usually preceded by severe life stress and are associated with significant impairment in occupational and social functioning (Drossman, Creed, Olden et al, 1999).

Studies have demonstrated that as an occupational group, farmers do not consult with physicians any more often than the general population (Simkin et al, 1998; Booth, Briscoe and Powell, 2000). Simkin et al (1998) carried out a survey of UK farmers and found that although 31% of farmers had health problems which interfered with their ability to work, the most common complaints were back problems and arthritis and not the symptoms profile reported by the exposed cohort in the present study. Indeed, agricultural workers in the present study do not share many of the characteristics of

patients with psychosomatic disorders except for the fact they suffer from a variety of physical symptoms. In many cases medical tests have found evidence of physical abnormalities.

The problem with concepts such as somatoform or psychosomatic disorder is that they are often applied as a diagnosis when physicians encounter something they do not understand. For example, a number of studies have followed up individuals who have been given a diagnosis of somatoform disorder and found that between 58-75% are subsequently found to have an underlying organic disease (Marsden, 1986). History has demonstrated that at any one time there are illnesses which are not recognized and dismissed as being psychological. There has been a clear decline in the rate of misdiagnosis over the past 50 years and the percentage of patients initially diagnosed with psychosomatic disorder and later identified as having an organic disorder has been decreasing (Moene, Landberg, Hoogduin et al, 2000; Stone, Smyth, Carson et al, 2005). This is partly explained by the development of more sophisticated diagnostic techniques and neuroimaging. In a recent study, Moene et al (2000) examined consecutive patients with a diagnosis of conversion disorder (referred for psychiatric opinion) and found 11.8% appeared to have a neurological disorder. However, it took a surplus of supplemental examinations before the final diagnosis could be made.

Compensation neurosis

All participants in this study were involved in litigation and the possibility exists that they exaggerated their difficulties for financial gain. Clinicians are often suspicious of the genuineness of impairments in individuals who are known to be claiming compensation. The following quote is a good example of this skepticism:

> *"A compensation neurosis is a state of mind, born out of fear, kept alive by avarice, stimulated by lawyers, and cured by a verdict" (Noy, 1975).*

In 1961, a distinguished Neurologist, Henry Miller published an article in the British Medical Journal on what he termed 'accident neurosis' He viewed with suspicion the psychological symptoms, frequently reported by patients who have been involved in accidents and suggested patients would recover on settlement of their claims.

Two broad issues make evaluation of effort important in the current study:
(1) Firstly, agricultural workers who took part in this study were involved in litigation and may have been motivated to under perform, whilst the control cohort were volunteers with no such incentive. Control subjects were paid a small fee (circa £25) to reimburse them for the time taken to complete the psychometric test battery and some may have felt more motivated than the exposed cohort to perform well. To minimize this risk, control subjects were told that payment was related to the time taken to complete the tests. They were not given the impression that payment was dependent upon good performance.

Differing levels of motivation in the control and exposed cohort are unlikely to account for the findings in this study because the differences remained significant with a few exceptions, even after removal of individuals from the exposed cohort who showed evidence of suboptimal performance. Furthermore, two psychometric test measures identified suboptimal performance in the control group, in addition to the exposed cohort, suggesting varying degrees of motivation in both groups.

(2) Power calculations based on previous studies suggest a sample size of sixty two individuals would be needed to have 80% power to detect a relationship between neuropsychological functioning and exposure history; yet significant differences in performance were found between the exposed and control cohorts in this study which numbered only 25 and 22 individuals respectively. This might reflect suboptimal performance in the exposed cohort. However, an alternative hypothesis is as follows: Previous studies have been carried out on individuals who were fit enough to be in employment and may have excluded individuals with more serious health problems who

may have retired or changed occupation. Seventeen individuals in the current study have retired on ill health grounds and the remainder has significantly reduced their working activities. Given the level of disability in this cohort, larger effect sizes might be anticipated between exposure history and cognitive function. Indeed, a major review of the literature regarding the effects of OPs on health was undertaken by a multi-disciplinary group of scientists in 1999 (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: COT). They concluded that the risk to health may have been underestimated by previous research because agricultural workers who had retired on ill health grounds had not been included in these studies. COT made it a requirement that future studies address this issue and include retired workers in their studies. Therefore, the fact that differences were found in the current study between relatively small samples of control and exposed individuals does not necessarily indicate that suboptimal effort explains these findings.

A number of researchers have investigated the validity of patient's complaints following accidents and brain injury. Lezak (2004) is of the opinion that most patients try to appear normal and minimize their deficits and that this is true of compensation claimants as well as those not seeking compensation. Some support for this view comes from the study by McKinlay, Brooks and Bond (1983) in which they found that the frequency with which a variety of physical, cognitive and emotional sequelae are found after head injury does not differ significantly between those cases in which a claim for financial compensation is being pursued and those in which it is not. Furthermore, some follow-up studies have found that contrary to popular belief, patients' symptoms do not necessarily resolve on settlement of their personal injury claims (Mendelson, 1982). A particularly relevant example of this is the finding of Reidy et al (1992) who examined a group of farm workers who were involved in litigation. Their symptoms did not improve following settlement of their claims.

However, the contention that faking is seldom a problem does not mean that it never arises (McKinlay and Kilfedder, 1992) and it may be more common in some contexts

than others such as in forensic settings (Bush, Ruff, Troster, Barth, Koffler, Pliskin, Reynolds, Silver, 2005). Questions about the validity of psychometric test scores may also arise when financial incentives for impaired performance exist, such as in the current study, whereby all of the exposed cohort were involved in litigation.
In clinical practice the determination of whether there are functional contributions to a patient's symptoms rests on a number of factors including (1) consistency of performance across tasks and over time (2) whether symptoms make medical sense (3) whether the profile of cognitive impairment makes psychological sense, for example the test profiles of fakers and real patients differ significantly in that fakers tend to perform poorly on all tests while real patients have no difficulty with some tests (McKinlay and Kilfedder, 1992) (4) an understanding of the patient's current situation, medical and social history, and (5) the results of tests known to be sensitive to functional complaints, yet few assessment tools are available for the accurate classification of those persons feigning cognitive and neuropsychological deficits (Rogers, Harrell and Liff, 1993).

Research has shown that the ability of skilled clinicians to detect feigned neuropsychological deficits when provided with standard data from the WAIS-R and WMS-R seldom exceed chance levels. Unfortunately, tests designed to assess malingering often lack sensitivity (Rogers et al, 1993). Therefore, a convergence of evidence from several sources is necessary before a clinician can determine a diagnosis of probable symptom exaggeration in a given individual (Miller, Ryan, Carruthers, Cluff 2004).

Four of the participants involved in the current study failed Rey's test of malingering, but this test has low reliability and validity in patients who are severely impaired. Poor performance is common in brain injured patients (Rogers et al, 1993) and for this reason it is not suitable for use with mentally dull individuals or those with neurological disease (Lezak, 2004). Rey's test was included in the current battery because it was assumed, given the findings of previous research concerning the effects of low-level exposure to OPs, that participants were unlikely to suffer from significant levels of impairment.

When patients are significantly impaired, it is inadvisable to rely on the results of one test and its cutting score when evaluating performance validity (Pankratz, 1988). Indeed research suggests that a combination of strategies produces the best classification rates, particularly those which include an examination of performance curves.

Analysis of performance curves in the current cohort produced conflicting results. Ravens method identified five individuals from the exposed cohort as showing suboptimal effort (only one of these individuals had been detected up by the Rey 15 item test) and four controls. This method has a high false positive rate so an alternative method of analysis was used which indicated that one of the exposed cohort and two of the controls was showing signs of inconsistent effort. Overall there was little agreement between the different methods used to detect incomplete effort and therefore little converging evidence for any one individual. Inconsistencies in performance could have been due to deliberate exaggeration, fatigue or depression. Seventy six percent of the exposed cohort was found to be suffering from clinically significant levels of depression and depression is known to reduce effort on cognitive testing.

Participants' performance on psychometric tests was re-analysed after removing the data from the four individuals who failed the Rey 15 item test, but this did not alter the pattern of results significantly. Differences between the exposed and control groups remained significant with a few exceptions.

The overall profile of performance on psychometric tests of individuals in the exposed cohort, was one in which they performed poorly on specific tests, whilst showing intact performance on others. This type of consistency is unlikely to occur by chance or to be seen in a cohort who is trying to simulate impairment. The fact that the performance of exposed and control groups in this study did not differ significantly on all psychological tests (i.e. performance on the majority of WAIS-R subtests was normal) adds strength to the argument that differences between the groups in this study are genuine, relating to specific types of cognitive function and are unlikely to be due to suboptimal effort.

Conclusion regarding differential diagnosis

To summarise, this study has identified a unique pattern of deficits in agricultural workers, which does not conform to that seen in CFS, solvent poisoning or mood disorder. Agricultural workers who took part in this study do not share many of the characteristics of individuals with psychosomatic disorder or malingerers. The pattern of deficits has much in common with that found in previous studies concerning the long-term effects of exposure to OPs. Agricultural workers report a close temporal relationship between symptom onset and OP exposure.

Limitations of the Present Study

This study has several weaknesses, which should be considered when interpreting the results.

Sample size

The number of participants in this study was relatively small and they were a self selected sample, yet their performance on neuropsychological tests was significantly worse than a group of healthy controls and significant correlations were found between exposure indices and tests of working and verbal memory. The results confirmed the findings of previous work, that there is a possible relationship between exposure to OPs formulations and poor performance on tests of information-processing speed, memory tests and mental flexibility. There is also an association between exposure to OPs and high rates of psychological distress.

Participants' exposure histories were found to differ enormously, despite the fact that they all appeared to have similar jobs. This is an important finding which has not been commented on before, no doubt because exposure history has not been examined in such detail. However, this factor needs to be taken into account by future research as it may account for some of the discrepancies noted in previous work. Given the shear number of factors, which can influence toxicity, it is important to obtain detailed information

about exposure history and to obtain large enough samples of participants to allow meaningful comparisons between homogeneous sub-groups.

It was impossible to determine the precise relationship between exposure history and cognitive function in this study because participants did not form a homogeneous group. Much larger sample sizes are needed to establish whether there are sub-groups of individuals who are more at risk than others of developing neurobehavioural problems following exposure to OPs (e.g. because they differ in terms of their exposure histories or genetically).

Sample bias

Population characteristics are usually inferred from measures taken from samples. If a sample is not truly representative of the population from which it is drawn (i.e. it is a biased sample) then it becomes impossible to make an accurate prediction about the population. Bias is reduced when subjects are randomly selected. Principles of randomness of selection are fundamental to theories of statistical inference, which are based on laws of probability and chance.

The participants in this study were not randomly selected. They are a self-selected sample of individuals who are pursuing personal injury claims against employers or manufacturers of OPs formulations. It is possible that some individuals may have been less effortful on cognitive tasks than others, but objective assessment of effort in this study yielded inconsistent and conflicting results. Future studies could avoid this issue by examining a more representative group of agricultural workers who are not involved in litigation.

To date, there are no reliable data regarding the prevalence of neurobehavioural problems in the farming community and their relationship to exposure history. It is important that future studies address this issue by examining randomly selected samples. A comparison between the current cohort and a randomly selected sample might be

useful in establishing whether there is anything unusual about them in terms of exposure history or genetic factors.

Attribution error and recall bias

It is possible that the participants in this study have made an attribution error in considering their ill health to be due to OP poisoning, when other factors may be involved in the aetiology of their symptoms. An important source of bias is recall bias where subjects attribute their difficulties to a publicized risk factor and exhibit better recall for evidence which confirms their bias than for evidence which contradicts it. Furthermore, all participants were involved in litigation and may have been motivated to attribute their symptoms to OP poisoning, or to exaggerate their symptoms for financial gain. The results of this study may reflect perception of risk as opposed to actual risk and further investigations are required of randomly selected cohorts who do not hold such strong views regarding the cause of their complaints and are not involved in litigation. Attempts were made to minimise the risk of attribution error such as reviewing medical records for alternative diagnoses and including a test of malingering. Review of participants' medical records excluded other aetiological factors other than exposure to OPs.

Exposure history and accuracy of recalled information

Related to the problem of recall bias, is the problem of accuracy of recalled information. Not surprisingly, it was difficult to obtain reliable information about exposure history retrospectively. A prospective cohort study would allow for more precise quantification of exposure, but may take a long time and be very expensive to complete. For example, it is difficult to determine how long the study period should be. If we use the findings of the current study as a guide, they indicate that exposure to OPs may need to take place on a regular basis for over 10-20 years before symptoms develop.

Prospective research designs are not ideal for the study of rare outcomes as it is necessary to study a very large number of individuals over time to obtain a sufficient

sub-sample of individuals with the condition of interest. In the absence of reliable epidemiological information regarding the prevalence of neurobehavioural problems in the agricultural community it is impossible to determine what sample size would be required for a prospective study. Other problems include the loss to follow-up of study participants involved in prospective studies, participants changing their habits over time or changing their practices as a result of surveillance. With regard to the former, the practice of sheep dipping has changed considerably over time. During the 1980s farmers were required to dip sheep twice a year and they used minimal amounts of protective clothing. Since the early 1990s farmers have been advised to use greater amounts of protective clothing and are now required to complete a course on the safe handling of OP formulations. Compulsory dipping is no longer in force and only takes place in response to an outbreak of sheep scab. If a prospective study were to be commissioned now and to include individuals who became involved in farming in the 1990s, results are likely to differ considerably from previous research.

Multiple comparisons

When multiple comparisons are carried out there is a risk of Type 1 error and this study used a large battery of psychometric tests. Statistical techniques were used to control for Type 1 errors and this did not alter the findings of this study. This indicates that significant differences in test performance observed between the control and exposed cohorts did not occur by chance. Furthermore, the exposed group did not perform poorly on all tests and this indicates that (1) effects occur in specific areas of cognitive functioning, and (2) the results are less likely to be explained by a bias operating across tests. Since the pattern of deficits seen in each exposed individual were similar and consistent, the deficits observed in the exposed cohort are likely to be real and not artifacts of study design

Study Strengths

The major advantage of small group, case studies is that they provide an opportunity to examine (1) rare conditions, and/or (2) specific issues in great depth.

It was possible to examine the nature and extent of neurobehavioural problems in this cohort in considerable depth, using clinically sensitive measures rather than administering brief screening tests which may lack sensitivity and/or specificity. The psychometric test battery was designed to cover a range of cognitive functions and participants were found to have deficits in particular areas whilst other abilities appeared intact. This is an important finding as some of the discrepancies noted in previous research may be due to limited test batteries being employed.

Exposure history was found to vary enormously despite participants appearing to have similar jobs, a factor which has not been commented on before, but may account for some of the discrepancies noted in previous work. Furthermore, the findings from this study suggest that restricting the definition of acute OP poisoning to that requiring hospital admission, may be misleading. Individuals may suffer from flu-like symptoms which have much in common with those associated with mild to moderate OP poisoning, but not seek medical treatment.

It was possible to obtain participants' medical records to exclude alternative diagnoses and this is seldom possible in large group studies or even in routine clinical practice.

Individuals who have retired from work were examined in addition to those who are still in employment and this is important as previous studies have focused on individuals who are fit enough to work. They may therefore underestimate risk.

Finally, the pattern of cognitive and emotional disturbance observed in this cohort is consistent with that seen in subcortical dementia. The cognitive dysfunction associated with subcortical dementia typically appears as slowed mental processing, impaired

concentration, executive dysfunction and memory disorder (primarily affecting retrieval rather than retention). Symptoms of cortical damage, such as aphasia, apraxia and agnosia are usually absent. Emotional disturbances usually involving apathy and depression are common; and motor symptoms are often the earliest sign of subcortical dementia (Brandt and Rich, 1995; Hodges, 1992). The OP cohort showed evidence of impaired verbal memory, executive dysfunction, reduced information-processing speed; and over 70% reported clinically significant levels of anxiety and depression. As far as the author is aware, the similarity between the psychological deficits observed in patients with subcortical dementia and individuals with a history of exposure to OPs has not been reported before, yet it may provide clues as to the mechanism and location of OP induced damage.

Implications for Future Research

The results of this study identify a number of areas for future research, some of which have been largely untouched by previous work:

Epidemiology and symptom specificity

The nature and extent of neurobehavioural problems in a representative group of individuals (i.e. not a self-selected sample) needs to be determined. To date, there is no reliable epidemiological information regarding the prevalence of ill health in agricultural workers who have been exposed to OPs. Comparisons should be made with unexposed populations and with other groups of neurological and psychiatric patients to determine which symptoms are specific to OP poisoning.

Vulnerable sub-groups

The possibility that there may be sub-groups of individuals who are more at risk than others needs to be considered. Factors which influence the toxicity of OPs need to be borne in mind by researchers and might include the nature of the OP compound, the route of exposure, the use of protective clothing or genetic predisposition of affected

individuals. Several investigators have suggested that genetic differences might place some individuals at greater risk of OP intoxication than others. Haley and Kurt (1997) examined 249 Gulf War veterans and attributed the symptoms of Gulf War illness to exposure to anticholinesterase poisons (e.g. OPs). A follow-up study of 25 of these veterans who reported ill health and neurobehavioural problems following active service (Haley, Billecke and La Du, 1999) identified genetic polymorphisms which might explain why some military personnel developed chronic illness and others with similar environmental exposures did not. These polymorphisms would have allowed more poison to enter neural tissue and increased the risk of neurotoxic damage.

Exposure history

Detailed information about exposure history needs to be obtained. Given that exposure history can vary enormously amongst individuals with similar jobs, future research will need to examine large samples of individuals to obtain sufficient numbers of subjects with similar exposure histories. Those who have been exposed dermally should be examined separately from those who are exposed by inhalation. Those with a history of dippers flu should be examined separately from those who do not. More information is needed about the content of different OP formulations and their solvent vehicles.

Test batteries

Internationally accepted, comparable and detailed psychological test batteries and medical tests should be employed as opposed to less well known or brief screening measures.

Prognosis and treatment options

Follow-up studies should be carried out to determine whether symptoms persist over time, improve or worsen. At present, there are no recommended treatment protocols for individuals who report chronic ill health following exposure to OPs, so there is a need for prospective treatment trials.

Mechanism of damage

The mechanism of action of OPs needs to be specified. For example, are the neurobehavioural problems associated with exposure to OPs the result of direct damage to the central nervous system or secondary to mood disorder or fatigue ? Comparison groups of CFS and psychiatric patients are needed to answer this question. A number of researchers have suggested that the mechanism by which OPs cause persistent neurobehavioural problems may not involve the cholinergic system. Pharmacological studies (i.e. involving drug challenges) of other neurotransmitter systems might shed further light on this issue.

Location of damage

Does the pattern of impairment in exposed individuals suggest a specific locus of damage? The pattern of impairment identified in the current study suggests specific areas of brain dysfunction involving subcortical structures, rather than generalised dementia. This has not been commented on before in agricultural workers. However, evidence of subcortical damage has been observed in other populations of individuals who have been exposed to OPs. For example, using Magnetic Resonance Spectroscopy, Haley, Marshall, McDonald et al (2000) found biochemical evidence of neuronal damage in different distributions in the basal ganglia and brainstem in Gulf War Veterans exposed to anticholinesterase poisons (e.g. OPs). Why might these areas be affected to a greater degree than other brain regions ? One explanation might be the fact that although acetylcholine neurotransmitters are distributed widely throughout the brain, activity is greater in some areas than others (Chaudhuri, Majeed, Dinan, et al, 1997).

Clinical implications

If further research confirms an association between exposure to OPs and neurobehavioural problems, this information needs to be disseminated to the medical community at large so that problems are detected early, patients are not given incorrect diagnoses and appropriate treatment strategies can be developed.

Final Summary and Conclusions

Since the 1950s there has been an increasing use of OPs for farming purposes throughout the world. Concern is growing about the problem of intoxication in those repeatedly exposed to OPs during farming operations. There is disagreement about whether prolonged low-level exposure causes chronic ill health due to inadequate exposure assessment, crude definitions of acute exposure and variations in methodology. This study has identified a range of cognitive and emotional problems in agricultural workers who were exposed to OPs pesticides on a regular basis for an average of 14 years. The possibility that dippers flu reflects undiagnosed acute poisoning is raised along with the suggestion that it may be misleading to classify agricultural workers as an occupational group who are exposed to low-levels of OPs, insufficient to cause intoxication. Indeed, the pattern of deficits identified in this cohort is consistent with that seen following acute poisoning. The OP cohort obtained lower scores on tests of memory, psychomotor speed, expressive language and mental flexibility in comparison to healthy controls. They were also found to suffer from significant levels of anxiety and depression. Although it is standard practice to take account of participants' depression scores when analysing their performance on neuropsychological tests, it may be inappropriate to do so in this case because the mood disorder seen in the OP cohort may reflect direct neurological damage following exposure to OP chemicals.

Statistical analyses revealed a number of associations between exposure variables and performance on specific psychometric tests, though some of these associations were confounded by background factors. The critical exposure variable remains unclear, but has been assumed to be level of exposure. However, there may be a complex interaction between exposure variables and individual susceptibility/vulnerability which may mean a direct dose response relationship between health and exposure history may not exist. Further research on a much larger, randomly selected sample of individuals is needed to explore this issue further. In 2004, the author was awarded a grant by the Department of Environment and Rural Affairs to explore some of these issues further.

CHAPTER SEVEN : REFERENCES

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APPENDICES

Appendix A

Ethical Approval & Informed Consent

THE JOINT UCL/UCLH COMMITTEES ON ETHICS OF HUMAN RESEARCH
COMMMITTEE A

RESPONSE FORM

DETAILS OF APPLICANT:

1. Name and Address of Principal Investigator: Sarah Jane Mackenzie-Ross
Sub-Department of Clinical Health Psychology
UCL
Gower Stree

---

2. Title of Project: Cognitive deficits associated with long term, low level exposure to organophosphate pesticides

---

3. Name and Address of Sponsor (if applicable): n/a

DETAILS OF LEAD REC: The joint UCL/UCLH Committees on Ethics of Human Research - Committee A

---

4. Name and address of REC: Research and Development Directorate, 1st Floor Vezey Strong Wing, 112 Hampstead Road, London NW1 2LT

5. REC Reference Number: 02/0226

Listed below is a complete record of the review undertaken by the Joint UCL/UCLH Committees on Ethics of Human Research, Committee A, with the decisions made, dates of decisions and the requirements at each stage of the review:

See letter dated 13th September 2002

THE FINAL DOCUMENTS AND ARRANGEMENTS APROVED BY THE LEAD REC:

The following items have been approved:

Ethics application form version July 2002
Information sheet version 2 dated August 2002
Consent form version 1 dated August 2002
Protocol

Date of Lead REC approval: 13 September 2002

Signature of Chair/Administrator: ..... Date: .....

Name (please print)

The Joint UCL/UCLH Committees on the Ethics of Human Research: Committees A and Alpha
The National Hospital for Neurology and Neurosurgery and the Institute of Neurology Joint Research Ethics Committee
The Eastman Dental Institute and Hospital Joint Research Ethics Committee

REC RESPONSE FORM

ETAILS OF APPLICANT:

Name and Address of Principal Investigator: Sarah Jane Mackenzie-Ross
Sub-Department of Clinical Health Psychology
UCL
Gower Street

Title of Project: Cognitive deficits associated with long term, low level exposure to organophosphate pesticides

Name and Address of Sponsor: n/a

ETAILS OF LEAD REC:

Name and address of REC: The Joint UCL/UCLH Committees on the Ethics of Human Research: Committee A; Research and Development Directorate, 1st Floor Vezey Strong Wing, 112 Hampstead Road, London NW1 2LT

REC Reference Number: 02/0226

sted below is a complete record of the review undertaken by the Joint UCL/UCLH ommittees on Ethics of Human Research, Committee A, with the decisions made, dates of ecisions and the requirements at each stage of the review:

ee letter dated 13th September 2002

HE FINAL DOCUMENTS AND ARRANGEMENTS APROVED BY THE LEAD REC:

ne following items have been approved:

hics application form version July 2002
formation sheet version 1 and 2 dated August 2002
onsent form version 1 and 2 dated August 2002
otocol

ate of REC approval: 13 September 2002

Senior
gnature of ~~Chair~~/Administrator: . **Date:** 20/11/02

ame (please print) **DOREEN SHARPE**

ur Ref: RM/WW/03A479

2 December 2003

The UCL/UCLH Joint Research Committee Alpha
Research & Development
1st Floor, Vezey Strong Wing
112 Hampstead Road
London NW1 2LT

Tel: 020 7380 9940
Fax: 020 7380 9937
Website: www.uclh.org

s Sarah Mackenzie Ross
ub-Department of Clinical Health Psychology
CL
ower Street

ear Ms Mackenzie Ross

EC Ref No: 02/0226 *(please quote in all correspondence)*
EC Name: Committee A *(please quote in all correspondence)*
udy Title: Cognitive deficits associated with long term, low level exposure to organophosphate pesticides

hank you for submitting a Progress Report Form, received on 24 November 2003, with regard to e above study. The Chair of the Joint UCL/UCLH Committees on the Ethics for Human Research: ommittee A, reviewed your Progress Report Form on 5 December 2003.

here are no ethical concerns and Chair has given approval for the study to continue for a further ar. The ethics committee will be notified at the next committee meeting on 22 January 2003.

HS RECs are compliant with the International Conference on Harmonisation/Good Clinical Practice CH GCP) Guidelines for the conduct of trials involving participation of human subjects.

our application has been given a unique reference number please use it on all orrespondence with the REC.

ours sincerely

endy Walker
hics Committee Administrator

ail: wendy.walker@uclh.org

nclosure: Progress Report Response Form

PROGRESS REPORT RESPONSE FORM

ll Title of Study: Cognitive deficits associated with long term, low level exposure to organophosphate pesticides

C Study No: 02/0226

te of REC approval: 17 October 2002

te of Submission: 17 November 2003

e REC has reviewed your Progress Report Form.

The REC has no comments:

The REC made the following decision

Ethics approval is granted for further year

te of REC Renewal of Approval: **12 December 2003**

gned Ethics Administrator:

nt Name: **Wendy Walker**

te of Report: **22 December 2003**

e form should be attached to the Progress Report form sent by you to the REC.

Sub-Department of Clinical Health Psychology

UNIVERSITY COLLEGE LONDON

GOWER STREET LONDON WC1E 6BT

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Clinical Tutor Team: 020 7679 1258
Senior Secretary: 020 7679 5699
UCL Switchboard: 020 7679 2000
Code from overseas: +44 20
Fax: 020 7916 1989

Please keep this for your records

Version 1 : August 2002 **CONFIDENTIAL**
Principal Investigator: Sarah Mackenzie Ross

Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

INFORMATION SHEET

Dear.....,

Re: Consent to use your test findings for research purposes

You came to see me on the ..... for a neuropsychological assessment, which involved a clinical interview followed by tests of memory and problem-solving ability. As you will recall, I prepared a medico-legal report for your solicitors, containing the results of this assessment.

Study purpose

I have now seen 21 agricultural workers who complain of ill health which they attribute to organophosphate poisoning and my colleague, Dr Julia Clark, has seen a further 12 people. I would like to collate everyone's results and summarise the findings in what is known as a 'group analysis'. This will enable me to determine whether people are suffering from similar neurobehavioural problems and how this might relate to their exposure to organophosphate pesticides (i.e. their exposure history).

I would like to include the findings of this group analysis in my doctoral thesis regarding the "Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides". I would also like to publish a summary of the results in a scientific journal at a later date. I am happy to send you a copy of any publications, which might arise from

this work. However, before I can proceed, I need your permission to include your test results in a group analysis along with general information about your exposure history.

Confidentiality and data security

All information will be kept confidential and an identification number will be used in place of your name. Sarah Mackenzie Ross will collect and store the data and only she will know the names of those individuals included in the group analysis. This information will not be made available to anyone else. Your name and address will not appear in Sarah's doctoral thesis or any scientific publication. Test results will be anonymous and presented like this 'case 1, memory test score 17'. Information regarding exposure history will be limited to general job descriptions (e.g. sheep dipper, animal health inspector, crop sprayer, horticulturist). Details about the average number of years people have spent working with organophosphate pesticides will also be provided, but it should not be possible to identify individuals from such general information.

What do you need to do ? - Consent

It is up to you to decide whether or not you agree to have your results included in a group analysis. You are not obliged to give consent and if you do not want to, this will not affect your legal rights.

I am keen to include the results of as many people as possible in the group analysis and would be very grateful if you would allow me to use your test results.

I enclose a consent form for you to sign, along with a stamped, addressed envelope for its return. I also enclose an additional copy of the consent form for you to keep, along with this information sheet.

Apart from signing and returning the consent form, you will not be asked to supply any further information. Sarah will only use information collected during your initial medico-legal assessment.

If you have any questions, please do not hesitate to contact me on 020 7679 1258. I work at UCL on Wednesdays and Fridays. Thank you for your attention.

Yours sincerely,

Sarah Mackenzie Ross
Consultant Clinical Neuropsychologist

Sub-Department of Clinical Health Psychology

UNIVERSITY COLLEGE LONDON

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Senior Secretary: 020 7679 5699
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Fax: 020 7916 1989

Please keep for your own records

Version 2 : August 2002 **CONFIDENTIAL**
Principal Investigator: Sarah Mackenzie Ross

Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

INFORMATION SHEET

Dear……,

Re: Consent to use your test findings for research purposes

You saw my colleague, Dr …… on …… for a neuropsychological assessment, which involved a clinical interview followed by tests of memory and problem-solving ability. As you will recall, a medico-legal report was prepared for your solicitors, containing the results of this assessment.

Study purpose

Dr Julia Clark saw a total of 12 agricultural workers who complain of ill health which they attribute to organophosphate poisoning and I have seen a further 21 people. I would like to collate everyone's results and summarise the findings in what is known as a 'group analysis'. This will enable me to determine whether people are suffering from similar neurobehavioural problems and how this might relate to their exposure to organophosphate pesticides (i.e. their exposure history).

I would like to include the findings of this group analysis in my doctoral thesis regarding the "Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides". I would also like to publish a summary of the results in a scientific journal at a later date. I am happy to send you a copy of any publications, which might arise from

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If you have any questions, please do not hesitate to contact me on 020 7679 1258. I work at UCL on Wednesdays and Fridays. Thank you for your attention.

Yours sincerely,

Sarah Mackenzie Ross
Consultant Clinical Neuropsychologist

Sub-Department of Clinical Health Psychology

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Please sign & return to Sarah Mackenzie Ross

Version 1 : August 2002

CONFIDENTIAL

CONSENT FORM

Study Title : Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

Principal Investigator: Sarah Mackenzie Ross

To be completed by the client:	**Please circle**
(1) I confirm that I have read and understood the information sheet dated August 2002 (version 1) for the above study and have had the opportunity to contact Sarah Mackenzie Ross and ask questions.	**Yes / No**
(2) I understand that Sarah Mackenzie Ross would like to include my psychological test results in a group analysis. I understand that general information about my exposure history will also be included in this group analysis.	**Yes / No**
(3) I understand that my name will be replaced with an identification number and test results will be anonymous. It will not be possible to identify individuals included in the group analysis, in any subsequent publications.	**Yes / No**
(4) I understand that Sarah Mackenzie Ross will present the findings of this group analysis in her doctoral thesis concerning the cognitive deficits associated with long-term, low level exposure to organophosphate pesticides.	**Yes / No**
(5) I understand that Sarah Mackenzie Ross may summarise these findings and publish them in a scientific journal at a later date.	**Yes / No**
(6) I understand that I am not obliged to give consent and this will not affect my legal rights.	**Yes / No**

I hereby give consent for Sarah Mackenzie Ross to include my psychological test results and general information about my exposure history, in a group analysis. I understand that the findings will appear in her doctoral thesis and may be summarised in a scientific journal at a later date.

Please circle **Yes** **No**

Please sign below and ask someone to witness your signature by signing this form as well. An example of a witness would be a spouse, relative, friend, doctor or your solicitor.

Name of Client (please print)	Date	Signature
Name of witness (please print)	Date	Signature
Principal investigator	Date	Signature

Sub-Department of Clinical Health Psychology

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Please sign and return to Sarah Mackenzie Ross

Version 2 : August 2002

CONFIDENTIAL

CONSENT FORM

Study Title : Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

Principal Investigator: Sarah Mackenzie Ross

To be completed by the client:	**Please circle**
(1) I confirm that I have read and understood the information sheet dated August 2002 (version 2) for the above study and have had the opportunity to contact Sarah Mackenzie Ross and ask questions.	**Yes / No**
(2) I understand that Sarah Mackenzie Ross would like to include my psychological test results in a group analysis. I understand that general information about my exposure history will also be included in this group analysis.	**Yes / No**
(3) I understand that my name will be replaced with an identification number and test results will be anonymous. It will not be possible to identify individuals included in the group analysis, in any subsequent publications.	**Yes / No**
(4) I understand that Sarah Mackenzie Ross will present the findings of this group analysis in her doctoral thesis concerning the cognitive deficits associated with long-term, low level exposure to organophosphate pesticides.	**Yes / No**
(5) I understand that Sarah Mackenzie Ross may summarise these findings and publish them in a scientific journal at a later date.	**Yes / No**
(6) I understand that I am not obliged to give consent and this will not affect my legal rights.	**Yes / No**

I hereby give consent for Sarah Mackenzie Ross to include my psychological test results and general information about my exposure history, in a group analysis. I understand that the findings will appear in her doctoral thesis and may be summarised in a scientific journal at a later date.

Please circle **Yes** **No**

Please sign below and ask someone to witness your signature by signing this form as well. An example of a witness would be a spouse, relative, friend, doctor or your solicitor.

Name of Client (please print)	Date	Signature

Name of witness (please print)	Date	Signature

Principal investigator	Date	Signature

Our ref: RM/cw/04A441

The Joint UCL/UCLH Committees on the Ethics of Human Research (Committee A)
Research & Development Directorate
1st Floor, Maple House
149 Tottenham Court Road
London
W1P 9LL

Ph: 0207 380 6977
Fax: 0207 380 9937

30th November 2004

Sarah Mackenzie Ross
Senior Clinical Tutor
Sub-Department of Clinical Health Psychology
University College London
Gower Street
London
WC1E 6BT

Dear Ms Ross,

Study title: *Cognitive deficits associated with long term, low level exposure to organophosphate pesticides*
REC reference: *02/0226*

Amendment date: *11th November 2004*

Thank you for your letter of 11th November 2004, notifying the Committee of the above amendment.

The proposed amendment to carry out the additional work requested and to continue this study for a further year was approved by the Chair on the 26th November 2004.

The Committee does not consider this to be a "substantial amendment" as defined in the Standard Operating Procedures for Research Ethics Committees. The amendment does not therefore require ethical review by the Committee and may be implemented immediately, provided that it does not affect the management approval for the research given by the R&D Department for the relevant NHS care organisation.

Documents received

The documents received were as follows:

1. Letter detailing proposed amendment. Dated: 11th November 2004.
2. Patient Information Sheet. Version 1: November 2004.
3. Consent Form. Version 1: November 2004.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

REC reference number: 02/0226	Please quote this number on all correspondence

Yours sincerely,

Caroline Williams
Committee Co-ordinator

E-mail: caroline.williams@uclh.nhs.uk

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Ms Caroline Williams
Ethics Administrator
The Joint UCL/UCLH Ethics Committee
Research & Development
1st Floor, Vezey Strong Wing
112 Hampstead Road
London NW1 2LT

11th November 2004

Dear Ms Williams,

REC Ref No: 02/0226
REC Name: Committee A
Study Title: Cognitive deficits associated with long term low level exposure to organophosphate pesticides.
Ethical renewal of approval granted December 2003

I am writing to update you with regard to the project detailed above and to request chairman's action/approval for a procedural amendment (please see below).

Project to date
Between 1999 and 2001 Sarah Mackenzie Ross (SMR) and a colleague (Julia Clarke) assessed over 30 agricultural workers who complained of ill health which they attributed to long term exposure to organophosphate pesticides. All of the agricultural workers underwent extensive psychometric testing and were interviewed about their occupational history, exposure history and social and medical background. Basic demographic information was also collected.

To date the project has involved the analysis of psychometric test results. In order to establish whether the performance of these agricultural workers on psychometric tests was normal or abnormal, SMR relied on published test norms rather than comparisons with a matched control group. Whilst this is routine in clinical practice, it is not ideal for research purposes.

Proposed change to study design
We now wish to recruit 24 healthy volunteers to act as a matched control group (matched for age, sex, and years in education). We would recruit study participants by placing and advertisement in 'Loot' magazine. To maximize recruitment, we will pay participants £6 per hour as we would require up to 4 hours of their time. Participants will be given an information sheet about the study and asked to sign a consent form (see enclosed). Participants will be asked to attend an appointment at UCL to undergo psychometric testing, involving tests of memory and problem-solving ability. They will be asked to complete questionnaires regarding their mood state at the time of testing and they will be interviewed about their previous exposure to pesticides. All information will be treated as confidential.

I would be very grateful if the ethics committee would (1) grant us permission to carry out this additional work and (2) extend approval for another year.

If you require any further information, please do not hesitate to get in touch.

Yours sincerely,

Sarah Mackenzie Ross MA. MPhil. C.Psychol AFBPsS
Senior Clinical Tutor, Doctorate in Clinical Psychology, UCL
Chartered Clinical Psychologist & Neuropsychologist

Sub-Department of Clinical Health Psychology
UNIVERSITY COLLEGE LONDON
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www.ucl.ac.uk/clinical-health-psycholo

Version 1: November 2004
Principal Investigator: Sarah Mackenzie Ross

CONFIDENTIAL

Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

INFORMATION SHEET

Dear Participant,

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Aims of the study
We have examined a number of agricultural workers who have complained of ill health which they attribute to long-term exposure to pesticides on the farm. In order to determine whether long-term exposure to pesticides does cause ill health, we need to recruit a group of healthy people who have not been exposed to pesticides over a long period of time, to act as a comparison group.

If I agree to take part in this study, what is involved?
If you agree to take part in this study, we will arrange a time for you to come in for a series of tasks which will involve test of memory and problem solving skills. You will also be asked to complete a brief interview about your lifestyle. In all, the session should take no longer than 4 hours and we will pay you £6.00 per hour. All information will be treated as confidential.

What will happen to the information I give the interviewer?
All information will be kept confidential and an identification number will be used in place of your name. The research team will collect and store the data and only they will know the names of those individuals who took part in the study. This information will not be made available to anyone else. Your name and address will not appear in any scientific publication. Test results will be anonymous and presented like this 'case 1, memory test score 17'. Data will be kept indefinitely.

What do I need to do? – Consent
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form, which we will give to you on the day. Participation is entirely voluntary and confidential and you are free to withdraw from the study at any point and without giving a reason.

All proposals for research with people are reviewed by an ethics committee before they can proceed. This proposal was reviewed by the UCL/UCLH Joint Research Committee A.

Thank you for taking the time to read this.

If you have any further questions about this study, please do not hesitate to contact one of our researchers:

Virginia Harrison
tel: 0207 6791902
e-mail: v.harrison@ucl.ac.uk

Kelly Abraham
tel: 0207 6791891
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Code from overseas: +44 20
Fax: 020-7916 1989
www.ucl.ac.uk/clinical-health-psycholog

Centre Number:
Study Number:
Patient Identification Number for this trial:

CONFIDENTIAL

CONSENT FORM

Version 1 : November 2004
Please sign and return to Sarah Mackenzie-Ross

Study Title: Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.

Principal Investigator: Sarah Mackenzie-Ross

To be completed by the client:

Please tick appropriately:

	Yes	No
I confirm that I have read and understood the information sheet dated November 2004 (Version 1) for the above study and have had the opportunity to contact the research team and ask questions		
I confirm that I have had sufficient time to consider whether or not I want to be included in this study		
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason		
I understand that Sarah Mackenzie-Ross will present the findings of this study in her doctoral thesis concerning the cognitive deficits associated with long-term low-level exposure to organophosphate pesticides		
I understand that the results of this study may be published but it will be impossible to identify individuals whom have taken part as their names will be replaced with an identification number		
I agree to take part in the above study		

1 form for Patient;
1 to be kept as part of the study documentation

Centre Number:
Study Number:
Patient Identification Number for this trial:

CONSENT FORM
Version 1 : November 2004

Study Title: **Cognitive deficits associated with long-term, low level exposure to organophosphate pesticides: An exploratory study.**

Principal Investigator: **Sarah Mackenzie-Ross**

Name of Participant (PLEASE PRINT)	Date	Signature
Name of Person taking consent	Date	Signature

Comments or concerns during the study

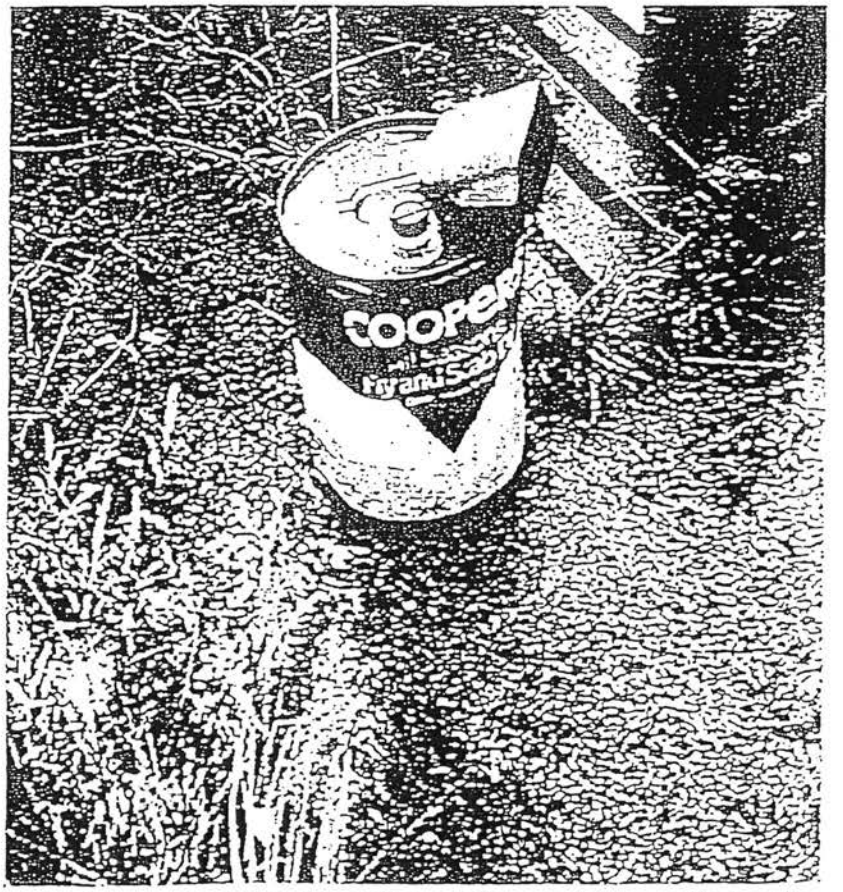
If you have any comments or concerns you may discuss these with the researcher. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Principle Investigator Ms Sarah Mackenzie-Ross at Sub-department of Clinical Health Psychology, University College London
(email: s.mackenzie-ross@ucl.ac.uk)
Please quote the Study Number and your Patient Identification Number at the top of this consent form.

1 form for Patient;
1 to be kept as part of the study documentation

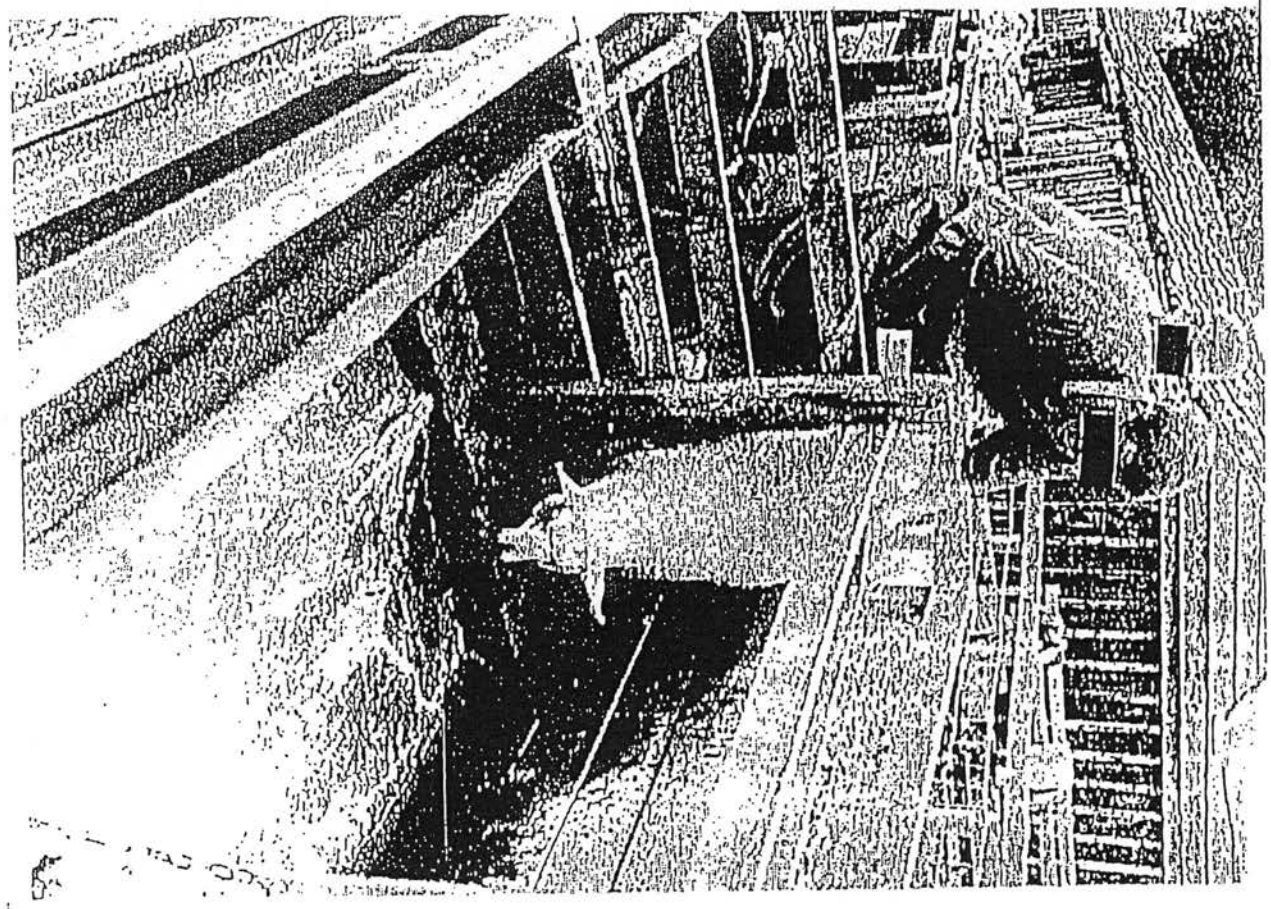
Appendix B

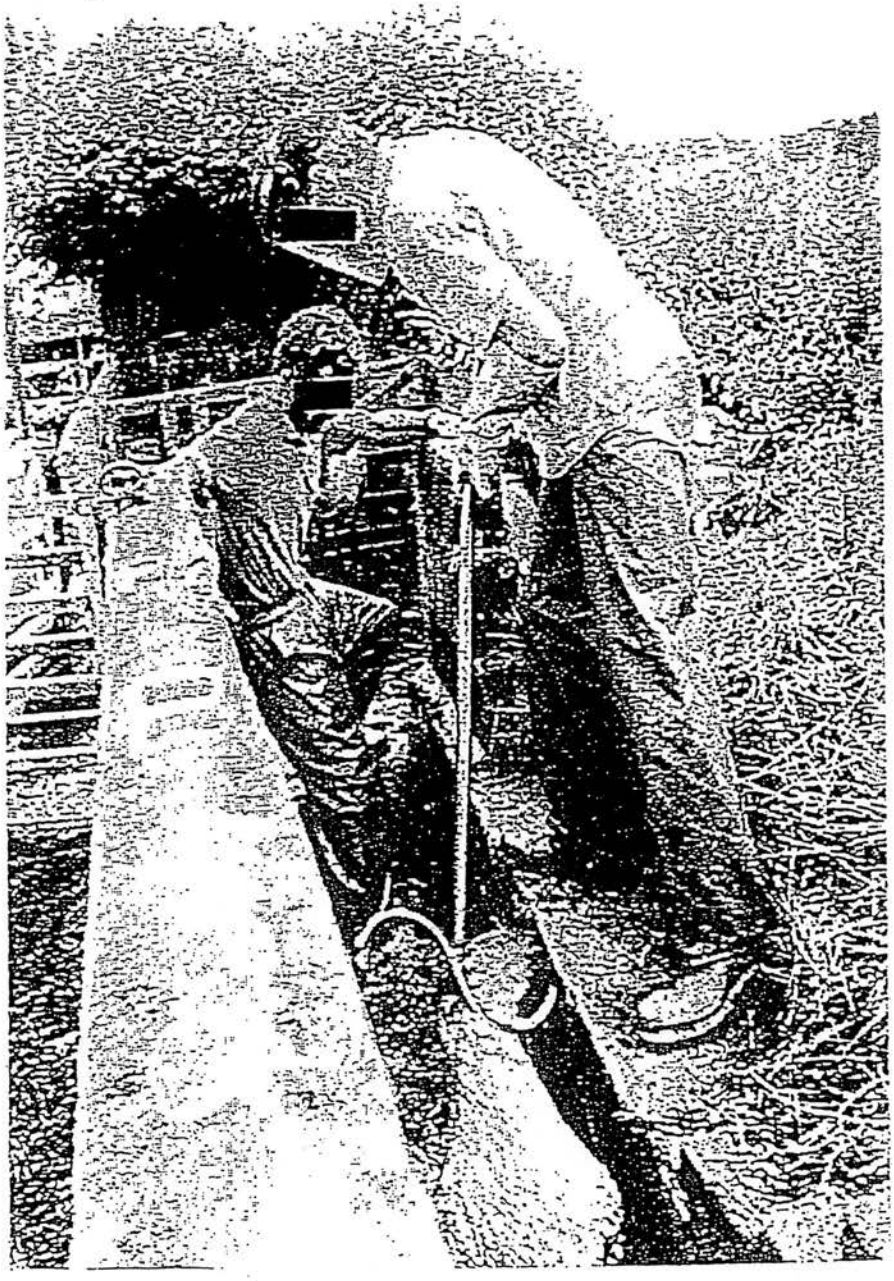
Photographs

The ring-pull cap has been removed and a screwdriver is used to lever the plastic spout out of the container (F24).



Residual concentrate on the surface of the container collected in the indentation below the carrying handle. This acted as source of contamination (FO1).





Appendix C

Measures

LIFE EVENTS SCALE

Below is a list of life events, which many people consider stressful, as they require people to make re-adjustments in their life. We would like to know whether you have experienced any of these over the last year. Please read down the list and put a tick beside any of the events you experienced in the **last year**.

Life Event	**Tick**	**Score**
Death of a Spouse		
Divorce		
Marital Separation		
Jail Term		
Death of a close family member		
Personal injury or illness		
Marriage		
Fired from Job		
Marital Reconciliation		
Retirement		
Change in health of a family member		
Pregnancy		
Sex difficulties		
Gain of a new family member		
Business re-adjustment		
Change in financial state		
Death of a close friend		
Change to different line of work		

Foreclosure of mortgage

Change in responsibilities at work

Son/daughter leaving home

Trouble with in-laws

Outstanding personal achievement

Wife begins or stops work

Begin or end school

Change in living conditions

Revision of personal habits

Trouble with boss

Change in residence

Change in school

Change in recreation

Change in church activities

Change in social activities

Change in sleeping habits

Change in eating habits

Vacation

Christmas

Minor legal violations

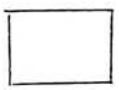
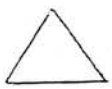
Other stressful life events not listed (please specify)

Memorization of 15 items

A B C

1 2 3

a b c

0  

1 11 111

THIS QUESTIONNAIRE REFERS TO DIPPING WITH

ORGANOPHOSPHATE COMPOUNDS ONLY (OPs)

NAME.....

DOB.....

TEL:.....

Occupation

Which of the following best describes your occupation (please tick):

A Farm Owner

A Farm Manager

A Farm Worker

Other (please explain).....

Frequency and Duration of sheep dipping

Between what years were you involved in dipping sheep using organophosphate compounds (OPs).....

How many times per year.....

How many days did each dipping session take to complete.....

On average, how many sheep did you dip with OPs – what was the flock size.....

Can you estimate how many days in total you have spent dipping sheep with OPs over your lifetime.....

When was the last time you engaged in sheep dipping with OPs.....

General Tasks

What % of time did you spend engaged in these tasks

Paddler (plunges sheep into bath).....%

Chucker (feed sheep into bath)).....%

Helper (herds sheep towards dipping area).....%

Which was your primary job.....

If you were involved in submerging sheep, what did you use (please circle)

Implement

Hands

Feet

Concentrate Handling

Did you work with sheep dip Concentrate (please circle) Y / N

Did you wear protective clothing whilst working with Concentrate Y / N

If yes what did you wear (please tick)

Gloves

Face shield

Waterproof leggings

Wellington boots

Apron

Were you involved in any accidents involving concentrate dip e.g. spillages Y / N

If yes, please give details.....

.....

.....

.....

Did you use meter systems to transfer concentrate to the bath Y / N

Have you been involved in cleaning or emptying the dipping bath Y / N

Can you recall the name of any products you used.....

.....

.....

Dipping Facilities

Type of bath (please tick)

Long swim
Short swim
Circular (with island)
Circular (no island)
Mobile
Other (specify)

Location of bath (please tick)

Outside / exposed
Outside / sheltered
Covered (open sides)
Within building
Within trailer
Other (specify)

Method of entry into bath (please tick)

Manual
Side entry (slip way)
Slope
Other (specify)

Did you have to replenish the bath Y / N

If yes, how many times per day.....

Protection

What protective clothing did you wear whilst dipping sheep

Gloves

Face shield

Waterproof leggings

Wellington boots

Apron

Was there a screen across the entry slope to deflect splashes Y / N

Were there waist high splash boards alongside the bath Y / N

Were there high sided screens at the exit of the bath Y / N

Were the draining pens away from the workers Y / N

Were the draining pen gates remotely operated Y / N

Were you ever : Splashed by sheep dip Y / N

If yes, how many times.....

.....

Soaked by sheep dip Y / N

If yes, how many times.....

.....

Did you ever fall into the bath Y / N

If yes, how many times.....

Other activities

Have you applied pesticides to crops Y / N

If yes, how often.....

Have you applied pesticides to grain Y / N

If yes, how often.....

Have you applied pesticides to animals Y / N

If yes, how often.....

Were you involved in sheep shearing

If yes, how many times.....

Dipper's Flu

Have you ever suffered from dippers flu Y / N

Can you estimate how many times have you suffered from dippers flu over your lifetime

.....

.....

Appendix D

Literature Review Search Strategy

Criteria for Considering Studies for this Literature Review

Types of studies

The main aim was to identify studies that had investigated the effects of organophosphate exposure on health, with or without control group comparisons. There were a great range of study types considered including epidemiological surveys, individual case studies, cross-sectional studies, before-after exposure studies and double blind comparison studies.

Types of participants

The main participant group of interest was those who had been exposed to organophosphate, whether or not they had been diagnosed with pesticide poisoning. Of specific interest were those who had been exposed to OPs as a result of farming or agricultural work.

Types of measures

The primary outcome measures of interest were in the following domains;
Neuropsychological function
Psychiatric features (i.e. psychiatric symptoms)
Behavioural measures
Self assessment of impact on daily living and quality of life (usually assessed via questionnaire)
Diagnosis of pesticide poisoning

SEARCH STRATEGY FOR IDENTIFICATION OF STUDIES

Database searches

The following search engines were used to identify appropriate studies:
PsycINFO
MEDLINE
Web of Science
Science Direct
Google Scholar

1. To begin with a general search was carried out to identify studies specifically looking at the effects of organophosphates on health. To do this a combination (using the AND and OR functions) of the following terms were entered into the various search engines: organophosphates, organophosphorus, organophosph*, health.

2. To narrow down the field and to look at relevant participant groups a combination (using the AND and OR functions) of the following terms were used: organophosphates, organophosphorus, organophosph*, farmers, farm*, agricultural workers, agricultur*.

3. To identify studies that considered the effects of chronic and/or acute exposure to organophosphates, a combination (using the AND and OR functions) of the following terms were used: organophosphates, organophosphorus, organophosph*, acute, chronic (farmers, farm*, agricultural workers, agricultur* were added at the end).

4. Studies which looked at the general prevalence of ill health in farmers (to act as a baseline comparison) were also identified. To do this a combination (using the AND and OR functions) of the following terms were used: farmers, farm*, agricultural workers, agricultur*, stress [EXPLODED], anxi*, anxiety [EXPLODED], depress*, depression [EXPLODED], chronic fatigue.

Reference searching
References cited at the end of all scientific papers identified by electronic search were reviewed in order to identify any studies that might have been missed by the search engines used.

Review articles
Existing review articles relevant to the topic of this study and Government Working Party Reports were examined for references to any further studies that may have been missed by the means mentioned above.