Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides

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PhD Thesis

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I, Sarah Mackenzie Ross, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

The aim of this thesis is to determine whether exposure to low levels of organophosphate pesticides (OPs) causes neuropsychological or psychiatric impairment. The thesis is arranged in three parts. Part 1 provides an introduction to neurotoxicology, the role of the psychologist, and the toxicity of OPs; Part 2 provides a historical review of the existing scientific evidence regarding the impact on human health of low level exposure to organophosphate pesticides. A major unresolved issue in the toxicity literature is whether repeated, low level exposure to OPs, in the absence of a history of acute toxicity, is harmful to human health. Part 3 presents the findings of a four year empirical study designed to address this issue. A cross-sectional study was undertaken in which the performance on neuropsychological tests of 127 sheep farmers with a history of low level exposure to organophosphate pesticides was compared with 78 non-exposed healthy volunteers (rural police workers) matched for age, gender, years in education and level of intelligence. Methodological weaknesses of earlier studies were addressed in the study design, such as inclusion of study participants who had retired on ill health grounds to take account of the 'healthy worker effect'; exclusion of study participants with a history of acute poisoning and those with a psychiatric or medical history that might otherwise account for ill health; and exploration of factors that may render some individuals more vulnerable to the effects of OPs than others (e.g. genetic differences in the capacity to metabolise and detoxify OPs). In the final chapter the findings are summarised and discussed, the study design is critically appraised and the implications of the findings are listed along with recommendations for future research.

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PART 1

An introduction to neurotoxicology, the role of the psychologist, and the toxicity of organophosphate pesticides.

Chapter 1 : An introduction to neuropsychological toxicology

1.1 History of toxicology

Toxicology is a multi-disciplinary science which draws on a number of disciplines (including psychology) to determine the adverse effects of chemicals in living organisms (xenobiotics). The discipline grew rapidly during World War II when the production of drugs and chemicals increased and expanded further in the 1960s following the thalidomide disaster, in which thousands of children were born with birth defects because their mothers were prescribed this sedative to control morning sickness during pregnancy. In 1962 the book Silent Spring, by Rachel Carson argued that pesticides were harming the environment, animals and humans. The book was widely read and resulted in growing recognition that many industrial chemicals (solvents, pesticides, fuels) and environmental pollutants are potentially damaging to animal and human health and that stricter regulations were needed to control the use of potentially toxic chemicals (Klaassen, 2008). Over the last forty years concerns have been raised about the possible effects of exposure to the increasing number of industrial and environmental chemicals being produced every year.

1.2 The scale of the problem

There are literally thousands of substances that possess the capacity to do harm in sufficient doses. It has been estimated that there are over 100,000 toxic substances in commercial use and approximately 2,300 new chemicals developed and submitted for registration every year (Environmental Protection Agency Office of Pollution Prevention and Toxics (OPPT), 2011; European Commission Joint Research Centre Institute for Health and Consumer Protection, 2011). The capacity of industry to produce chemical substances outstrips research which means our knowledge regarding the health effects of many substances is limited. This gives rise to differences in opinion and controversies regarding the effects of a number of substances.

1.3 Toxicity testing and safety standards

Although regulatory bodies require premarket toxicological testing in animals, the adequacy of toxicity testing is open to question since it is designed to characterise the

toxic effects of chemicals, not to demonstrate their safety. There is no set testing procedure with standardised tests that chemicals must pass to allow their commercial use, rather a tiered approach is used in which the testing protocol is determined by (1) consideration of the predicted substance effects given the chemical structure of the product and (2) findings from initial, short-term, animal studies. Toxicological testing is based on the relatively short-term administration of a single active ingredient in animals whereas human exposures are often to complex mixtures of compounds over a lifetime. There are a wide range of potential health effects which could be studied (mortality, carcinogenicity, reproductive effects, physical ill health, neurological symptoms, neurobehavioural symptoms) but financial constraints, concern for the welfare of animals and methodological issues mean not all of these outcomes are studied (Klaassen, 2008). Few chemicals are subjected to behavioural analysis and the neurotoxicity of many substances has never been tested (Hartman 1995). Animal testing alone is not sufficient to protect public health as epidemiological studies suggest many products which pass basic screening tests have the capacity to affect human health (Alavanja, Hoppin & Kamel, 2004). In order to gain a thorough understanding of the toxic effects of chemical substances, it is essential to carry out human studies (e.g. case studies, epidemiological studies, clinical trials of new pharmaceutical products). Different species may have different responses to particular chemicals and an infrequent effect may only become apparent in a large population study as opposed to a limited number of animal experiments (Klaassen & Watkins, 1999).

Establishing safe exposure standards for many substances is fraught with difficulty and depends on the outcome of interest (e.g. acute toxicity, chronic ill health, reproductive or developmental effects etc). In an ideal world we would know the dose/response relationship for each potential effect, but this is seldom the case. Recently important questions have been raised about the validity of exposure standards as many are changed as new information becomes available. Lead is a classic example of this. Over time it has come to be seen as harmful at lower levels than originally thought, and more recently it has been suggested that there is no safe level of exposure to lead (Hartman 1995; Schwartz, 1994). To complicate matters further, it is extremely difficult to establish a safe standard that will protect everyone due to inter-individual differences in susceptibility and genes which affect our ability to metabolise chemicals. In addition the possible synergistic effects of chemical combinations are rarely assessed (i.e.

chemical interactions) despite the fact that exposure to chemicals rarely occurs in isolation.

1.4 Detection and health care professionals

Many people are exposed to harmful substances in the workplace (e.g. farm workers, chemical plant workers, laboratory workers, motor mechanics, other transport industry workers, painters) but exposure to neurotoxic substances can also occur in the home (e.g. to carbon monoxide, paints/solvents, pesticides). Some individuals will experience symptoms of acute toxicity or develop chronic ill health but few will attribute their symptoms to toxic exposure unless they have been advised of the risks associated with chemical products they are working with (Hartman, 1995). To complicate matters further, few health care professionals receive training in toxicology and are unlikely to consider a toxic cause for a patient's symptoms. This is especially problematic as the results of routine medical examinations are frequently normal as many toxic substances are rapidly metabolised and excreted from the human bloodstream (Hartman, 1995). This means a definitive diagnosis for patient's complaints may never be reached. Neuropsychology is a discipline which has an important role to play in evaluating patients and establishing an explanation for their symptoms. Indeed, neuropsychological testing has been described as the most sensitive means of examining the effects of toxic exposure (Lezak, 1984).

1.5 The role of neuropsychological research

Many chemicals can interfere with nervous system function and chemical injury often results in cognitive, emotional and behavioural change (Hartman, 1995). For this reason the neuropsychologist has an important role to play in detecting and evaluating the effects of neurotoxic substances. Neuropsychological tests can be used to assess impairment after exposure to toxic substances known to affect cognitive functioning and they are often included in research studies to contrast neuropsychological abilities in exposed and unexposed populations. They also have the advantage of being relatively inexpensive, non-invasive, portable, objective, reliable, sensitive and capable of detecting signs of neurotoxic damage in the absence of other neurological signs (Berent & Albers, 2005; Hartman 1995; Lezak, Howieson & Loring 2004). However, neuropsychological assessment has its limitations. Test results in isolation are non-

specific, and the neuropsychologist must make inferences beyond the test results to reach conclusions. They need to consider evidence from other disciplines and they need to be aware of specific issues which are pertinent to the field of toxicology.

1.6 Common issues in neuropsychological toxicology

1.6.1 Proving a link with exposure

Establishing a causal link between neuropsychological impairment and exposure to neurotoxic substances is not easy. Patients frequently present long after exposure has ceased and the toxic substance has been eliminated from the body which means objective evidence of exposure (i.e. a biomarker) is seldom available (Hartman, 1995). Instead, the clinician has to rely on the patient's history to determine whether they have been exposed to a neurotoxic substance, the likely amount they have been exposed to (dose), how often (frequency) and over what time period (duration). Unfortunately patient's testimony can be unreliable because of the limits of human memory, and processes such as recall bias and attribution error. Often the most that can be achieved is to document the opportunity for exposure and evidence suggestive of a causal relationship, such as the timing and onset of symptoms, biological plausibility and specificity of symptoms (Berent & Albers, 2005).

1.6.2 Spectrum of effects

Neurotoxic substances can cause generalised damage involving many different bodily systems or highly selective damage to particular regions (Karalliedde, Feldman, Henry & Marrs, 2001; Klaassen & Watkins, 1999). Neurotoxic substances may injure the nervous system directly by damaging dendrites, axons, myelin, neurons and supporting cells or by interfering with neurotransmission; or they may have indirect effects on other organs such as the liver and kidneys resulting in a build up of toxic substances and metabolites in the body (Karalliedde et al, 2001; Klaassen & Watkins, 1999). They may also interfere with other processes such as immune system function, protein synthesis, energy conversion, oxygen transport and gene expression. Hence, they usually give rise to a host of non-specific symptoms which are similar to those associated with other degenerative and metabolic illnesses. The neuropsychologist needs to consider whether symptoms make medical sense given the mechanism of action of specific chemicals (i.e are symptoms biologically plausible) and must rule out alternative diagnoses before

proposing a toxic cause for a patients symptoms (Berent & Albers, 2005).

1.6.3 Acute vs chronic effects

Neurotoxic substances have immediate effects on the nervous system, but some are capable of producing additional delayed effects days or weeks later such as the organophosphates and carbon monoxide (Karalliedde et al, 2001; Penney, 2008). For many chemicals the toxic effects of a single, acute, high level exposure are known from animal testing or case studies involving incidents of severe poisoning (Klaassen & Watkins, 1999). In contrast, less is usually known about the effects of repeated, low level exposure, which may produce quite different effects from high level exposure as the mechanisms underlying acute and chronic effects may differ. As a consequence, considerable controversy surrounds the possibility that chemicals which have passed basic screening tests may be harmful in low or repeated doses.

1.6.4 Individual differences

Considerable inter-individual differences exist in the capacity to metabolise and detoxify certain chemicals and therefore some individuals may be more effected by lower doses than others and symptom profiles and/or severity of symptoms may vary amongst patients (Berent & Albers, 2005; Hartman, 1995, Klaassen, 2008). Children and the elderly may be at greater risk than young adults; there may be gender differences in the ability to detoxify certain substances (e.g. alcohol); and genetic differences between individuals in terms of their capacity to metabolise certain substances (e.g. organophosphates). Finally, individuals in particular occupations such as farming, painters, transportation workers, and electronic workers, may be at greater risk than others (Karalliedde et al, 2001; Klaassen, 2008; Penney, 2008). These factors can alter the severity of the toxic response and lead to variations in symptom profile and severity that frequently confuse healthcare professionals and researchers (Berent & Albers, 2005; Klaassen, 2008).

1.7 Concluding remarks

Despite these difficulties, the neuropsychologist has an important role to play in both clinical and research settings when it comes to evaluating the effects of toxic substances (Lezak at al, 2004; Lucchini, Albini, Benedetti & Alession, 2005). Many substances interfere with nervous system function and therefore psychological processes (Hartman,

1995). Neuropsychology has devised standardised tests, capable of detecting subtle abnormalities associated with neurotoxic exposure and neuropsychologists are trained in scientific method which means they are able to use scientific methodology and a systematic approach to address the various clinical and research challenges inherent in neurobehavioural toxicology.

The primary objective of this thesis is to determine whether evidence exists to support the view that long-term, low level exposure to organophosphate pesticides is associated with the development of chronic neurobehavioural problems. Organophosphates are increasingly used around the world for a variety of agricultural, domestic, military and industrial purposes and concerns have been raised about the effects of these chemicals on human health. The next chapter summarises the development, consumption and toxicology of organophosphates (OPs) and discusses factors that influence toxicity which need consideration by clinicians and researchers.

Chapter 2 : Organophosphates

2.1 Development and consumption of organophosphates

Organophosphate pesticides were derived from World War II nerve gas agents (sarin, soman, tabun) and were modified during the 1950s and 1960s to have selective toxicity to insects and lower toxicity to mammals (Karalliedde et al, 2001; Klaassen, 2008). They have since become the most widely used group of pesticides / insecticides in the world and are extensively used in agriculture, horticulture and veterinary medicine, and for domestic purposes to control insects around the home and garden. They are also used for public hygiene purposes to control insects in public buildings such as offices, schools and hospitals, and they are even used on commercial aircraft travelling to and from tropical countries. OPs continue to be used by the military as both chemical weapons and pesticides; and they are also used by industry as solvents, plasticizers, flame retardants and extreme pressure additives (lubricants). This means a very large number of people will be exposed to these chemicals in some form during their lifetime, and questions have been raised about the effects OPs may have on human health (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) Report 1999; Karalliedde et al, 2001; Royal Colleges of Physicians, Psychiatrists and General Practitioners Report, 1998).

Demand for OP pesticides has increased over the last three decades because they are kinder to the environment and have less biopersistence than their predecessors (i.e. the organochlorines; Karalliedde et al, 2001). However, concern about the effects of these pesticides has been growing with their increasing use throughout the world, but there is a lack of reliable epidemiological data on the impact of OP pesticides on human health. It is estimated that approximately 3-5 million pesticide poisonings occur annually worldwide and pesticide poisoning is around 13 times more likely in developing countries than highly industrialised ones (Baxter, Adams, Aw, Cockcroft & Harrington, 2000; Karalliede et al, 2001; Rosenstock, Keifer, Daniell, McConnell & Claypoole, 1991; World Health Organisation (WHO), 1990). More people are at risk of long term, low level exposure but cases of adverse effects may not be reported unless affected individuals recognise their symptoms are due to pesticide exposure and seek medical help (Ecobichon & Joy, 1994; European Centre for Ecotoxicology and Toxicology of

Chemicals (ECETOC) Report, 1998; WHO Report, 1990).

2.2 Organophosphate use in the UK

In the UK, OPs have been used in agriculture, horticulture and veterinary products to control sheep scab, blow fly, ticks and warble fly. They were first licensed for use in the 1970s and sales dramatically increased during the 1980s following the introduction of compulsory sheep dipping to control sheep scab, an infectious disease which spoils fleece (Stephens, 1996; Watterson, 1999). Scab mite was eradicated in the UK in the 1950s but reappeared in the 1970s. As a result, the Ministry of Agriculture, Fisheries and Food (MAFF) made it compulsory to dip sheep twice a year between 1984-1988 and once a year between 1988-1991. Compulsory dipping was discontinued in 1991. The most common OP compounds used in sheep dip formulations at that time were diazinon, propetamphos and chlorenvinphos. Between 1985 and 1998 over 600 reports of ill health following exposure to sheep dip were received by a government 'adverse reaction surveillance scheme' (Dunn, 2002; Tahmaz, Soutar & Cherrie, 2003). In 1999 an organophosphate working group was commissioned by the Department of Health to review the available evidence concerning the toxicity of OPs and hear evidence from sheep farmers regarding symptoms of ill health attributed to exposure to sheep dip. The committee concluded that evidence exists to suggest acute poisoning from OPs can result in ill health, but the possibility that low level exposure causes similar effects is unproven (COT report, 1999) and recommended the UK government commission further research to address this issue.

2.3 Process of dipping

Sheep dipping is a process whereby sheep are immersed in a bath (either indoors or outdoors) which has been filled with pesticides to eradicate parasites. Sheep dip is purchased by the farmer as a concentrate which needs to be diluted before use. Farm workers prepare the dipping bath by filling it with water and concentrate and there is a high risk of skin contamination during this process (Institute of Occupational Medicine (IOM) Report, 1999). The dip bath is replenished several times during the day so farm workers may handle concentrate on more than one occasion during the day. Several individuals are involved in the process of dipping. Sheep are gathered and forced into the bath (the worker who does this is often referred to as a 'chucker') and a farm worker

usually submerges the sheep under the water using an implement (this person is called a 'paddler') and may be splashed by sheep dip as the animal struggles. Some protective clothing is usually worn, such as wellington boots, waterproof leggings and rubber gloves, but gloves frequently disintegrate after brief use because OP formulations based on organic solvents and containing phenols are liable to penetrate clothing and degrade rubber unless washed off properly (IOM Report 1999). 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 risk ingesting OPs (IOM, 1999).

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 also potentially hazardous (letter from the Chief Vet at Coopers Animal Health, 1988).

2.4 Health effects

Farmers refer to a condition of general malaise which often follows sheep dipping, which they call 'dippers flu' (headaches, aching limbs, runny nose, nausea, tightness of chest, diarrhoea, increased sweating and salivation). It follows the time of dipping and can last for up to 48 hours. Rees (1996) measured the incidence of symptoms following exposure to sheep dip in a group of 24 farmers in the UK. 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. Subsequent studies of UK sheep farmers utilising different methodologies (including case series analyses, postal questionnaires and clinical evaluations) found many individuals complain of persistent ill health long after exposure has ceased including neuropsychological and neurological abnormalities, mood disorder, headache, chronic fatigue and weakness (Ahmed & Davies 1997; Beach et al, 1996; Davies, Ahmed & Freer, 2000; Dunn, 2002; Jamal, Hansen & Julu, 2002; Mackenzie Ross, Clark, Harrison & Abraham, 2007; Pilkington et al, 2001; Solomon,

Poole, Palmer, Peveler & Coggon 2007; Stephens et al, 1995; Tahmaz et al 2003). However the results of these studies caused much controversy and debate as to whether OP pesticides were responsible for these symptoms. Sheep farmers are considered to have relatively low level exposure to OPs and whilst it is generally established that high level, acute exposure to OPs can cause short-term adverse effects (mediated by cholinergic excitation which disrupts nervous system function), the possibility that long-term, low level exposure to OPs may cause ill health remains controversial (COT Report, 1999; Royal Colleges' Report, 1998).

2.5 Toxicology of organophosphate pesticides

An OP is a chemical compound which contains both carbon and phosphorous. There are a large number of OP chemicals with a diverse range of chemical structures and activities, but OP pesticides all have in common the ability to inhibit acetylcholinesterase (AChE). Human exposure to OP pesticides can result from eye or skin contact, inhalation or oral intake and may be accidental or intentional (i.e. suicide). Organophosphates are absorbed rapidly through the skin, lungs, gastrointestinal tract and conjunctiva (Karalliedde et al, 2001; Klaassen, 2008). Once absorbed, OP pesticides inhibit the enzyme acetylcholinesterase (AChE) which is responsible for metabolising acetylcholine (ACh), an important neurotransmitter in the central, peripheral and autonomic nervous systems. Inhibition of the enzyme results in accumulation of acetylcholine at synapses and over-stimulation of nerves and muscles, resulting in a constellation of acute symptoms, followed by paralysis of transmission if supplies of ACh become depleted (Ecobichon and Joy, 1994; Karalliedde et al, 2001; Royal Colleges' Report, 1998). OPs do not only bind to AChE but also other esterases and their inhibitory impact on esterases can result in four syndromes, the acute cholinergic crisis, the intermediate syndrome, organophosphate delayed polyneuropathy and chronic neurobehavioural damage. These syndromes can occur independently of one another and may have different underlying mechanisms (Abou-Donia, 2005).

2.5.1 Acute poisoning – the cholinergic crisis

Characteristic symptoms of mild poisoning include fatigue, headache, weakness, dizziness, sweating, wheezing, coughing and have been well documented (Baxter et al, 2000; COT Report, 1999; ECETOC Report, 1998; Royal Colleges' Report, 1998; WHO

Report, 1990). The symptoms are not unlike influenza and are frequently not recognised as being caused by exposure to OPs. Moderate poisoning may result in abdominal cramps, nausea, vomiting, muscular tremors, bradycardia, lowered heart rate and blood pressure; and severe poisoning may cause respiratory difficulty, cardiac arrest, convulsions, coma and possibly death.

Recovery from mild poisoning usually occurs within 24-48 hours and 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. OPs are capable of producing several delayed physical and neurological syndromes.

2.5.2 The Intermediate Syndrome

The intermediate syndrome (IS) may follow successful treatment of an acute cholinergic crisis 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. The syndrome is reported fairly frequently in developing countries (Senanayake & Karalliedde, 1987).

2.5.3 Organophosphate induced delayed polyneuropathy

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 cord 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 containing two OPs that can cause OPIDN (chlorpyrifos and coumaphos) were available until 1989 and 1991 respectively.

2.5.4 Chronic neurological and neurobehavioural changes

Neurobehavioural changes involving subtle cognitive impairment, greater psychiatric morbidity and chronic fatigue have been reported (Ahmed & Davies, 1997; Davies, Ahmed & Freer, 1999; Hartman, 1995; Karalliedde et al, 2001; Klaassen, 2008; Mackenzie Ross et al, 2007; Ray, 1998a; 1998b; Solomonet al, 2007; Stephens et al, 1995). 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; Fatehyab-Ali, Hassan & Tariq, 1979), inhibition of other enzymes and proteins (Jamal, 1997; Pancetti, Olmos, Dagino-Subiabre, Rozas & Morales, 2007; Pope, 1999) and apopotic neuronal cell death (i.e. programmed cell death involving free radical generation and oxidative stress; Abou-Donia, 2005; Kapur, Radotra, Minz & Gill, 2007). The research evidence concerning neurobehavioural changes following exposure to OPs will be discussed in greater detail in the next chapter of this thesis.

2.6 Factors influencing toxicity in humans

2.6.1 Measurement of exposure

Accurate estimation of severity of exposure is critical for the validity and power of studies investigating the adverse effects of OPs. A large number of factors have been identified which can influence the toxicity of OPs and efforts should be made to collect data on as many of these variables as possible (Karalliedde et al, 2001; Klaassen 2008). The particular OP compound an individual is exposed to and the level/duration of exposure are frequently assumed to be the most relevant/critical variables (Karalliedde et al, 2001; Klaassen 2008). There are a large number of OP pesticides of differing chemical composition and although all have some toxic effects in humans, these can vary widely. Other important factors which influence toxicity include route of exposure (i.e. oral, dermal, inhalation), concentration and duration of exposure, rates of metabolism and elimination of OPs from the human body. However it is extremely difficult to obtain reliable information regarding these variables in human occupational

exposures as objective indices of exposure, such as external monitoring of contaminants in the atmosphere and/or internal biological monitoring of workers (to determine level of exposure) is extremely rare in most occupations (Karalliedde et al, 2001; Klaassen 2008). Biological monitoring (i.e. urine and blood analysis of OP metabolites) is of limited value in studies of long-term health effects as the human body rapidly metabolises and eliminates toxins, making biological monitoring useful for assessing severity of recent but not long-term exposure. Often the most that can be achieved is a rough estimate regarding level and duration of exposure based on an individual's testimony / self report regarding the number of years they have worked with a specific chemical product, how frequently they used it and over what time frame. Given the limits of human memory, exposure information collected in this way may be unreliable.

Some researchers create job exposure indexes in an attempt to consider all of the different aspects of exposure history which may be relevant (such as those listed above: Buchanan et al, 2001; Cherrie & Robertson, 1995; London & Myers, 1998; Stewart, Prince, Colt & Ward, 2001). Exposure metrics vary greatly in terms of which variables they consider important and range from simple methods whereby different measures of frequency and duration of exposure are multiplied together to provide an overall rating; to more complex formulae which attempt to estimate intensity of exposure in addition to duration by incorporating weightings for variables such as job activity, use of protective clothing and so on. Exposure metrics do not reflect absolute exposure but provide a ranking within a population under study. They are often considered to be an improvement over simple measures of exposure such as ever/never been exposed or duration of exposure, but the validity of such measures is dependent on the assumptions underlying the metric, the variables considered, the weighting assigned to variables and the accuracy of information provided by respondents.

The inability of clinicians and researchers to acquire precise information about dose, frequency and duration of exposure probably explains, at least in part, the continuing debate regarding the relative contribution these variables make in producing toxic effects. A major unresolved issue in the toxicity literature is whether repeated, low level exposure to OPs is harmful to human health (COT Report, 1999). Although it has been established that acute, high level exposure is harmful, it remains unclear whether chronic health problems are a result of a history of acute exposure (dose) or frequency

and duration of low level exposure (cumulative damage acquired over time). In addition, there is no clear cut definition of 'high level' and 'low level' exposure and studies purporting to be examining the effects of low level exposure may include people with a history of acute poisoning. At present, the definition of high level exposure revolves around whether individuals seek medical help (COT Report, 1999), yet research has shown that variables other then severity of illness determine whether individuals consult physicians (Pitts & Phillips, 1991). A definition of this kind may under-estimate instances of acute poisoning as medical health seeking behaviours are lower in rural communities (British Medical Association, 2005).

2.6.2 Route of exposure

Route of exposure influences the severity of toxic effects and varies across occupations. For example, orchard workers and crop sprayers apply pesticides by spray, and are at greater risk of inhalation exposure than sheep dippers who are at greater risk of dermal penetration (Buchanan, et al, 2001). Having said that, some workers may be exposed by more than one route, for example sheep farmers who engage in several roles such as dipping and herding sheep into containers and who smoke/eat when working are at risk of dermal, oral and inhalation exposure. Dermal and oral absorption result in less toxicity than inhalation as OPs undergo a degree of metabolism in the gut and liver before reaching the systemic circulation following these routes of exposure. In contrast, absorption following inhalation is rapid and almost complete as the OPs pass into circulation without having been metabolised by the liver (Karalliedde et al, 2001).

2.6.3 Rates of metabolism, individual differences & interactions with other chemicals

Following uptake, OPs are distributed widely throughout the body. Although they are lipophilic and have the potential to form depots in fat, skin and bone tissue (Karalliedde et al, 2001), they are generally degraded rapidly and most of the products are excreted in urine within about 2 days (Klaassen, 2008). This makes it difficult to obtain biological markers of exposure from blood or urine samples, unless an individual is examined shortly after exposure. Metabolism of OPs involves a number of enzyme systems which usually make the pesticide more water-soluble and easier to excrete but in some cases metabolism increases toxicity (Costa & Furlong, 2002; Klaassen, 2008). To complicate matters further several of the enzymes involved in metabolising OPs are involved in metabolising other substances which means their ability to metabolise OPs

may be altered if an individual is exposed to compounds that share the same metabolic pathway of detoxification such as certain prescribed medicines, by dietary factors (e.g. alcohol, grapefruit juice) or exposure to other industrial chemicals at the same time as OPs (Abou-Donia et al, 1996; Costa & Furlong, 2002; van Himbergen, et al, 2008).

Genetic predisposition can influence an individual's capacity to metabolise OPs as there is considerable individual variability in the activity of enzyme systems involved in the detoxification of OPs. For example, paraoxonase (PON1) is a liver and plasma enzyme which contributes significantly to the detoxification of OPs and may be a useful biomarker of individual susceptibility to OP toxicity. Paraoxonase activity shows wide differences within Caucasians and other ethnic groups and a subgroup (40-50%) of the UK and US population have low activity with 4% of the population being deficient in this enzyme (COT report 1999; Costa et al, 2000; Mackness et al, 2003; Richter & Furlong, 1999). Hence an individual's response to exposure may be affected by polymorphisms in genes involved in pesticide metabolism (Cherry et al, 2002; Costa et al, 2003; Mackness et al, 2003).

Polymorphisms of PON1 have been found to modulate the toxicity of OPs in animal studies (Costa & Furlong, 2002). In human populations, two common polymorphisms have been identified in the coding sequence of PON1: L55M and R192Q. Costa and Furlong (2002) found that there are large inter-individual differences in level of plasma PON1 activity such that individuals with the same genotype may have different levels of protection. Therefore, it is important to measure the level of protein expressed in an individual's plasma in addition to determining genotype. Cherry et al (2002) and Mackness et al (2003) investigated the relationship between PON1 genetic polymorphisms and activity levels in farmers reporting chronic ill health attributed to OP exposure in sheep dip (cases) and sheep farmers who carried out similar activities but remained well (controls). They found that the PON1 192 polymorphism was more common in people reporting ill health and that these individuals were more likely to have the R or L PON1 alleles than similarly employed controls who believed themselves to be healthy. Furthermore, cases were more likely than referents to have low serum hydrolytic activity for diazoxon. Indeed, Mackness et al (2003) reported that farmers in the lowest quintile of diazoxon hydrolysis were 2.5 times more likely to report ill health (which they attributed to sheep dipping) than farmers in the highest

quintile. These findings suggest that PON1 status (i.e. genotype and PON1 level) is an important biomarker of individual susceptibility to OP toxicity and an important consideration for any future studies seeking to address the health effects of OP exposure.

2.6.4 Use of protective measures and cross cultural differences

The risk of exposure to chemical substances can be reduced by the use of protective clothing, but many workers, particularly those in developing countries, cannot afford personal protective equipment (PPE) and/or find it unbearable to wear in hot humid conditions. Many UK sheep farmers found PPE impractical to wear and in some cases PPE actually exacerbated exposure for example when rubber gloves became degraded by the solvents and phenols in pesticide formulations which allowed OP pesticides to penetrate the gloves and become trapped against the skin layer, thereby prolonging dermal exposure. Legislation regarding the use of pesticides and minimisation of health hazards varies considerably between developed and developing countries, with the most stringent legislation occurring in the USA where some states stipulate that agricultural workers wear protective clothing and are biologically monitored during the course of their work (Karalliedde et al, 2001). In contrast workers in some developing countries are driven by poverty to work long hours and may be unable to read instructions regarding the dilution, application, storage and safe disposal of pesticides. Many are exploited by employers and compelled to use equipment and pesticides provided with total disregard of precautionary measures (Karalliede et al, 2001).

2.6.5 Outcome measures

As mentioned in Chapter 1, there are a range of potential health effects following exposure to OPs (mortality, carcinogenicity, reproductive effects, physical ill health, neurological symptoms, neurobehavioural symptoms) but financial constraints, concern for the welfare of animals and methodological issues mean not all of these outcomes are studied (Klaassen, 2008). There are a large number of OP pesticides which differ in their chemical composition and capacity to produce some of these effects making it hard to form an overall view on the toxicity of certain compounds as it depends on the outcome of interest.

2.7 Concluding comments

OPs have complex effects on the human body and are capable of producing a variety of acute and chronic effects. There is a need for further research as millions of workers and an unknown number of individuals will be exposed to OPs at some point in their lives. Outstanding issues remain in the toxicity literature such as whether repeated, low level exposure to OPs, in the absence of a history of acute toxicity, is harmful to human health. Unfortunately, only a crude definition of high versus low level exposure exists and quantification of exposure is fraught with difficulty. Level of exposure is frequently assumed to be the only biologically critical variable, but this chapter has reviewed several other variables which can influence toxicity. Future researchers need to account for these variables in their study design. The next chapter reviews the available scientific evidence concerning the effects of long-term, low level exposure to OPs and illustrates the methodological issues and challenges facing researchers.

PART 2

Neurobehavioural problems following low level exposure to organophosphate pesticides.

A systematic & meta-analytic review.

Chapter 3 : Neurobehavioural problems following low level exposure to organophosphate pesticides: A systematic review.

3.1 Background

Pesticides prevent millions of people from starving to death and from disease, but they are harmful to humans under certain circumstances. Organophosphate pesticides are the most widely used insecticides in the world and are considered by the World Health Organisation to be one of the most hazardous pesticides to vertebrate animals, responsible for many cases of poisoning worldwide, particularly in developing countries where adequate protective measures are lacking (De Silva, Samarawickrema & Wickremasinghe, 2006; WHO report 1990). Concern about the effects of organophosphates on human health has been growing as they are increasingly used throughout the world for a variety of agricultural, domestic and industrial purposes. The neurotoxic effects of high level acute poisoning are well established and involve inhibition of the enzyme acetylcholinesterase (AChE) causing changes in peripheral, autonomic and central nervous system function (cholinergic crisis; see previous chapter for more detail). However, the possibility that long-term low-level exposure to OPs in doses below that causing acute toxicity causes ill health is controversial.

A number of researchers have addressed this question using a variety of different methodologies and populations, but previous research has produced inconsistent findings, with some studies finding evidence of ill health and cognitive impairment following low level organophosphate exposure while others have not (see reviews by Alavanja et al 2004; Arcury & Quandt 1998; Colosio, Tiramani & Maroni, 2003; COT Report 1999; De Silva et al 2006; ECETOC Report 1998; Kamel & Hoppin 2004; Mearns, Dunn & Lees-Haley, 1994; Ontario College of Family Physicians (OCFP) Report 2004; Ray, 1998a; 1998b; Royal Colleges' Report, 1998). Major methodological differences may account for these inconsistencies such as examination of different occupational groups with different levels and routes of exposure, use of protective clothing, from different cultural backgrounds examined over different time periods (e.g. following a single episode of exposure, several years of exposure or over a lifetime).

Since many more individuals are likely to be at risk of long-term, low level exposure, rather than acute poisoning it is important to get a clear answer to the question of

whether low level exposure is harmful to human health. The aim of this chapter is to review the available evidence concerning the neurotoxicity of long-term, low level exposure to organophosphate pesticides. A systematic review of the literature was undertaken using Medline, Embase and Psychinfo databases without date limitation. A large body of literature exists concerning the neurotoxicity of OPs incorporating different methodologies, populations examined and outcome measures, so strict inclusion and exclusion criteria were applied to limit the review to relevant, high quality studies of human adult populations.

3.2 Methods

3.2.1 Objectives

The overall aim of this chapter is to review and evaluate the available evidence concerning the neurotoxicity of long-term, low level exposure to OPs in order to determine whether low level exposure to OPs is associated with neurobehavioural dysfunction.

3.2.2 Criteria for considering studies for this review

There is a large body of literature concerning the neurotoxicity of OPs including animal studies, single case-studies, group studies, questionnaire and telephone surveys, studies which have included objective clinical examinations, retrospective and prospective studies. Subtle differences in study aims influence the selection of study participants. For example, some studies have examined the effects of acute poisoning or the chronic health effects which may follow a prior history of one or more episodes of acute poisoning; whilst others have investigated the short-term effects of a single season of pesticide use in individuals who may or may not have a history of prior acute intoxication; or the consequences of long-term, low level exposure in the absence of a history of acute intoxication. Different study participants have been selected including children, adults, individuals from industrialised and developing countries, individuals from different occupational groups with different routes of exposure. Different outcomes have been evaluated, such as mortality, pathology, physical symptoms (e.g. chronic fatigue), reproductive outcomes, cancer, neurotoxicity, behaviour. These different methodologies are not strictly comparable and probably account for the inconsistent findings of previous research.

This review will focus on the effects of low level exposure to OPs on neurobehavioural function in the absence of a history of acute poisoning. The review will not include studies concerning the neurobehavioural effects that may follow one or more episodes of acute poisoning. This review will also limit itself to neurobehavioural effects on human adult populations. Studies concerning children and adolescents will not be included as developmental issues complicate interpretation of neurobehavioural data. Children may be particularly susceptible to the effects of toxic substances because of their developing nervous system (Hartman, 1995). This review will be limited to studies which meet the following criteria.

Table 3.1 Inclusion/exclusion criteria for studies in this review

Inclusion criteria	*Exclusion criteria*
Studies of OP exposure	Pesticides other than OPs
Effects of long-term, low level exposure in the absence of an episode of acute poisoning.	Immediate or long-term health effects following acute poisoning.
Long-term exposure over a lifetime	Short-term, acute effects following a single season of pesticide use
Observational group studies of human adults	Animal studies, studies of children, single case reports
Neurobehavioural outcome measures	Only used outcome measures which are not neurobehavioural eg carcinogenicity, mortality
	Outcome measures which involve symptom questionnaires rather than objective measures.
	Non-English language papers

3.2.3 Search methods for identification of relevant studies

Relevant studies from the 1960s onwards were identified from MEDLINE, EMBASE and PsycINFO databases (via Ovid interface) using both subject headings and textword search strategies on the 27th August 2009 (please see Appendix 1 for full details). Retrieved articles were reviewed to evaluate title and abstract content and to eliminate articles that were not relevant for this review and to remove duplicates. Government

working party reports, relevant textbooks and references cited at the end of articles were also examined to ensure all relevant material was included in this review.

3.2.4 Data synthesis and quality assessment

Details of relevant studies were entered into summary tables showing study objectives, study populations, exposure and outcome measures. Study methodology varies considerably so the following factors were taken into consideration when evaluating studies:

1. Does the study design adequately address the question of whether long-term, low level exposure to OPs has adverse effects on neurbehavioural function - is the study design appropriate for the stated research question?

2. Does the study provide adequate information concerning the exposure history of study participants?

> Does the study evaluate the effects of exposure to organophosphates or does it concern exposure to a mixture of pesticides, including OPs?
>
> Does the study evaluate the effects of long-term, low level exposure to OPs in the absence of a history of acute exposure?
>
> Does the study include participants with a history of acute exposure? If so, do they take this into account in their analysis by analysing these individuals as a separate group?

3. Does the study evaluate human, adult populations and if so, from which country, cultural and ethnic backgrounds were the study participants from?

4. Was a suitable, matched comparison group of unexposed individuals examined?

5. Were objective, reliable, valid, standardised, outcome measures included?

3.3 Results

3.3.1. Numbers of articles retrieved from database searches

A total of 562 articles were identified as potentially relevant by the three databases. Appendix 1 shows the number of articles identified by the different databases according to search strategies used (MEDLINE identified the greatest number of potentially relevant articles). The titles and abstracts of these articles were subsequently reviewed and 483 were excluded for the following reasons: the article concerned a pesticide which was not an organophosphate, the article concerned the effects of acute OP poisoning, the outcome measures were not neurobehavioural, study populations included children, adolescents or animals, the article concerned the treatment of OP poisoning or the benefits of biological monitoring or protective clothing; the article was a review article rather than an original research study (the latter were reviewed thoroughly however, particularly the reference lists cited at the end of these articles to ensure all relevant papers were retrieved). After removing articles which had been duplicated between the three databases and search strategies a final sample of 36 articles were selected and reviewed thoroughly. A further 7 studies were added to the review which had not been identified by the database searches. These articles were noted in the references cited at the end of other articles. This left a final sample of 43 original articles for review (please see Appendix 1 for study details and sources).

3.3.2 Excluded studies

The first step of the review process was to determine whether all 43 articles selected from the initial screening of titles and abstracts, met inclusion criteria for this review. This was not always apparent from a review of titles and abstracts. Sixteen studies were excluded following this second stage of the review because they did not meet the inclusion criteria listed in Table 1 (see Appendix 1 for further details).

A further twelve studies were excluded because the study design did not adequately address the question of whether long-term, low level exposure to OPs impairs neurbehavioural function. The literature concerning this issue encompasses considerable variation in study methodology. It is possible to group studies according to design and three broad study designs are apparent in the literature; (1) epidemiological studies which use proxy measures of exposure such as occupational group (2) pre/post episode

or season of exposure evaluations (3) epidemiological studies which provide quantitative information about exposure history. However the first two study designs do not adequately address the issue of whether low level exposure to OPs is harmful. Hence, six studies were excluded because they used proxy measures of exposure such as occupational group or residency in a particular geographical region and although they found evidence to suggest a link between farm work and the development of ill health causality could not be determined (Beseler et al 2006; Browne et al 2006; Cole et al 1997; Kamel et al 2003; Parron, Hernandez & Villanueva, 1996; Rohlman et al 2007). Assumptions were made that deficits identified related to pesticide exposure, but in all of these studies participants were exposed to a wide range of pesticides making it difficult to determine whether adverse effects relate to a single pesticide such as OPs or the use of pesticides in combination. Dose-response relationships could not be determined and the influence of variables which do not relate to exposure such as lifestyle or stress, couldn't be ruled out. For this reason, studies which used proxy measures of exposure do not appear in this review.

Some studies have attempted to address the issue of whether chronic exposure to OPs causes ill health by examining workers before and after an episode or season of pesticide use (Albers et al, 2004; Bazylewicz-Walczak, Majczakowa & Szymczak, 1999; Daniell et al, 1992; Maizlish, Schenker, Weisskopf, Seiber & Samuels, 1987; Misra, Prasad & Pandy, 1994; Rothlien et al, 2006; Salvi et al, 2003). The advantage of pre/post season study designs is that they allow a more detailed analysis of dose-response relationships to be made than other study designs and they are particularly useful for determining whether (1) a single episode of exposure affects health (2) symptoms persist, worsen or resolve over time (Salvi et al, 2003) and (3) evaluating the utility of biological monitoring and the relationship between biological markers of exposure and onset of symptoms.

However, most fail to address the issue of whether long-term, low level exposure to OPs causes ill health and are therefore beyond the remit of this review. The exception is the study by Bazlewicz-Walczak et al (1999) in which two types of analyses were undertaken, both pre and post season evaluations looking for change in performance over time, but also comparisons of exposed and unexposed cohorts prior to the spraying season, matched on important variables which might otherwise affect cognitive

function. The latter analysis is crucial for establishing whether cumulative, low level exposure is the causative factor, since any cohort comparisons undertaken following spraying seasons may simply pick up immediate, acute effects of exposure. This study was retained in the meta-analysis.

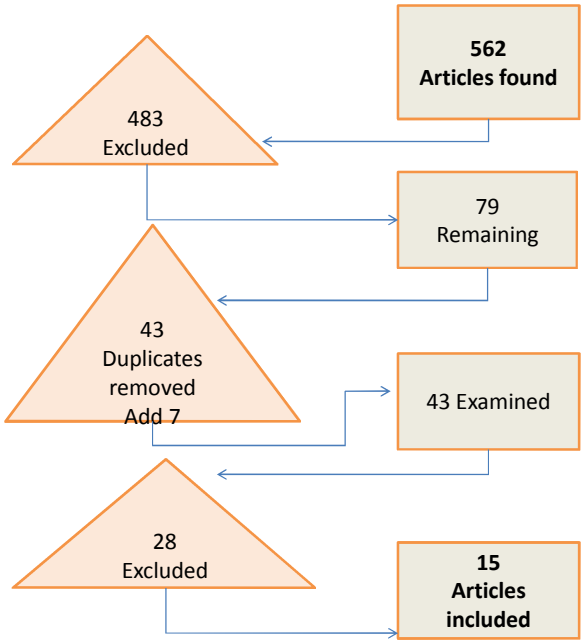


Figure 3.1 Number of potentially relevant articles identified during initial literature searches, exclusion and inclusion figures.

3.3.3 Findings of the review: Epidemiological studies that provide quantitative measures of exposure

Fifteen epidemiological studies were identified as being suitable for inclusion in a meta-analysis. All addressed the issue of whether long term, low level exposure to OPs is associated with neurobehavioural deficits, but different populations of people were examined including chemical plant workers, greenhouse workers, pest control operatives, pesticide applicators (sheep dippers, fruit tree sprayers, crop sprayers). Study participants came from both developed and developing nations. They were exposed to a range of different OPs and duration of exposure ranged from an average of 2 years to over 20 years.

This review will now describe these studies. They will be grouped according to the occupational status of study participants and country of origin because level and route

of exposure varies between jobs and in developing and developed nations. Details are also provided concerning the neurobehavioural measures used in the study and whether the control subjects were matched on important variables, known to affect performance on cognitive tests, such as age and years in education. All of this information is summarised in Tables 3.2.

3.3.3.1 Chemical plant manufacturers

3.3.3.1.1 Developing countries

Srivastava et al (2000) examined 59 Indian workers recently exposed to different chemicals during the manufacture of 'quinalphos' and 17 control subjects. Groups were matched for age and sex, but controls were more educated than exposed 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 nervous system 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 would be expected to outperform the exposed cohort. A further criticism concerns the limited amount of information provided about exposure history.

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 the American Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R; American Psychiatric Association, 1994) and completed the General Health Questionnaire. 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.

3.3.3.2 Pest control operators

3.3.3.2.1 Developed countries

Steenland 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. Groups were matched for age and sex, but controls were more educated than exposed subjects. 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. The main limitation of this study is the fact that study participants had a relatively short history of exposure to OPs.

3.3.3.3 Farm workers & pesticide applicators

3.3.3.3.1 Developed countries

Rodnitzky, Levin and Mick (1975) studied 23 farmers and commercial pesticide applicators in Iowa who regularly used OP compounds (and had done so within 2 weeks of testing), but were asymptomatic and compared them with 23 non-exposed farmers. Mean plasma AChE levels were within normal limits (but slightly lower in exposed farmers) but the groups did not differ significantly on tests of memory or reaction time (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 and lifetime exposure history of study participants was not provided and the sample size is very small.

Ames, Steenland, Jenkins, Chrislip and Russo (1995) examined 45 Californian pesticide applicators 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. The groups were not matched for age or education, the exposed cohort being older and less educated. Subjects underwent nerve conduction studies, vibrotactile sensitivity tests, a test of postural sway and eight neuropsychological tests of psychomotor speed, attention, fine motor control, memory and mood state. No evidence of neurobehavioural problems was found in the exposed cohort and the authors conclude that neurological sequelae can be prevented by avoiding acute poisoning. However, no information is provided about exposure history other than the fact workers had been exposed to cholinesterase inhibiting pesticides and the duration of time workers were exposed to pesticides is unclear.

3.3.3.3.2 Developing countries

Farahat 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 (Benton, Sivan, des Hamsher, Varney & Spreen, 1994). 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 change in blood AChE activity and that psychometric assessment is a useful method for the early detection of chronic effects of OP pesticide exposure.

3.3.3.4 Fruit Tree Sprayers

3.3.3.4.1 Developed countries

Stephens and Sreenivasan (2004) looked at the neuropsychological effects of long-term low level exposure to OPs in 37 English orchard sprayers, none of whom had a history of acute poisoning. Their performance on 7 neuropsychological tests was compared with 26 pig farmers and 31 construction workers, matched for age and education. Pig farmers had a history of exposure to pesticides. Orchard sprayers (and pig farmers) differed from unexposed construction workers in terms of the time taken to complete negative statements of the ACTS syntactic reasoning test. However, psychometric test findings did not correlate with the index of cumulative exposure used in this study, but the authors suggest this may be due to measurement error inherent in the index.

Fiedler, Kipen, Kelly-McNeil & Fenske (1997) compared 57 fruit tree sprayers in New Jersey (with no history of acute poisoning resulting in hospitalisation) with controls. Groups were matched for age, but controls were more educated and had higher reading scores than the exposed subjects. The exposed cohort had slower reaction time (although age predicted some of the variance in RT scores), but no other differences between the groups on neuropsychological testing were found. 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.

3.3.3.4.2 Developing countries

London, Myers, Nell, Taylor and Thompson (1997) looked at the neurobehavioural effects of long-term, low level exposure to OPs by examining 163 African fruit tree sprayers and comparing them with 84 unexposed labourers. Groups were matched for age, years in education and levels of illiteracy. Neuropsychological tests had to be adapted for the study population due to cultural differences reported by previous studies which influence performance on standard tests and because participants had little formal education. Nine percent had a history of acute poisoning with OPs and over eleven percent had a history of exposure to other neurotoxic chemicals. Alcohol consumption was high. Nine controls had a history of pesticide exposure through agricultural work. Small occupational effects were observed on two out of seven tests but may have been the result of multiple comparisons. The authors suggest the failure to find significant association between exposure and neurobehavioural performance may have been a result of exposure misclassification or the fact that workers with poor neurobehavioural performance may have quit their jobs and not been included in the study. Cross cultural issues make this study very difficult to interpret.

3.3.3.5 Greenhouse workers

3.3.3.5.1 Developed countries

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.

Roldan-Tapia et al (2006) examined the effects of different degrees of pesticide exposure on neuropsychological performance. Data from 24 acutely poisoned workers and 40 non-poisoned but chronically exposed Spanish greenhouse sprayers were compared to 26 controls. Groups were matched for education but the low exposure

group was significantly younger than the other two groups. The pesticides used included OPs and carbamates. Chronically exposed subjects were split into two subgroups, high exposure (more than 10 years handling pesticides) and low exposure (less than 10 years handling pesticides). Acutely poisoned subjects had been poisoned in the last 3 months and required treatment in the local hospital at the time of poisoning. Neuropsychological assessment found evidence of reduced visuo-motor , perceptual and constructive abilities, verbal learning and speed of processing and increased rates of anxiety. Subjects with high chronic exposure had similar neuropsychological profiles whilst those with low chronic exposure were similar to controls.

Bazylewicz-Walczak et al (1999) sought to determine the behavioural effects of chronic exposure to OPs by examining 51 women employed in gardening enterprises and compared them to 25 unexposed controls, matched for age, years in education, smoking and alcohol use. None of the exposed subjects had a history of acute poisoning. Psychological examinations were carried out before and after the spraying season using the Neurobehavioural Core Test Battery recommended by the WHO. No deterioration in cognitive or emotional function was found after one spraying season. However, exposed and unexposed cohorts differed on both occasions with OP exposed subjects showing slowing of perceptuo-motor functions and reported a higher degree of anxiety, depression, irritability, fatigue and memory problems. The authors conclude that a single season of pesticide use may not cause immediate behavioural effects, but repeated low level exposure to OPs over extended periods of time may produce chronic neurobehavioural effects.

3.3.3.6 Studies of UK Farmers

In the UK, a number of studies have been carried out of sheep farmers who used organophosphate pesticides to destroy parasites on sheep. Farmers were required by law to dip sheep once or twice a year between 1976 and 1991. A number of individuals reported ill health following dipping which they attributed to exposure to OP pesticides. Although previous studies undertaken in the UK suggest a link between exposure to sheep dip and the development of neurobehavioral problems, it is unclear whether this is due to a history of acute poisoning or a result of cumulative low level exposure.

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 current review and was reported by **Jamal et al (2001).** 74 individuals who participated in phase 2 were classified into three groups according to whether they had signs of peripheral neuropathy ('no', 'possible' and 'probable/definite' signs) 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 may be affected. The study design is unusual in that it assumes 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 peripheral nerve damage and central nervous system damage 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 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, speed of information processing and syntactic reasoning (a finding replicated by Stephens and Sreenivasan, 2004). 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 nervous system, 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 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. Indeed, **Beach et al (1996)** followed up 20 of these farmers and split them into two groups according to how many symptoms they reported after dipping. The 10 most symptomatic and 10 least symptomatic farmers then underwent a neurological examination several months after dipping and were compared to 10 unexposed controls. Although the prevalence of neurological abnormalities was low amongst the farmers, subtle adverse neurological effects were detected involving two point discrimination in the hands and feet and calf circumference. Stephens, Spurgeon and Berry (1996) also investigated whether a relationship exists between acute symptoms suffered immediately after dipping and the development of chronic neurbehavioural problems later. However, they did not find any evidence of an association and they suggest chronic neurobehavioural effects occur independently of acute symptoms of exposure.

Mackenzie Ross et al (2007) compared 25 farm workers with a history of apparent low level exposure to sheep dip with 22 non-exposed healthy volunteers on neuropsychological tests. Two thirds of farm workers had retired or reduced their workload on ill health grounds and all were involved in litigation. They performed significantly worse than non-exposed healthy volunteers on tests of mental flexibility, response speed and memory; and over 70% suffered from mood disorder. Although this study included participants who had retired on ill health grounds, the sample size was small and self selected making it unclear how representative they are of the farming community as a whole. Furthermore, many farm workers appeared to have a history of undiagnosed acute poisoning.

3.3.3.7 Summary

The 15 epidemiological studies described in this chapter have produced inconsistent results. Twelve out of fifteen studies reviewed found evidence of neurobehavioural impairment following long-term, low level exposure to OPs, ranging from subtle deficits in one or more areas (usually reaction time and fine motor control: Fiedler et al, 1997; London et al, 1997;Steenland et al, 2000; Stephens et al, 2004) to major deficits in several cognitive domains (memory, attention, reaction time and visuo-spatial

deficits; Bazylewicz-Walczak et al, 1999; Farahat et al, 2003; Mackenzie Ross et al, 2007; Roldan-Tapia et al 2005 and 2006; Srivastava et al, 2000; Stephens et al, 1995). Emotional difficulties were also frequently reported (Amr et al, 1997; Farahat et al, 2003; Mackenzie Ross et al, 2007; Steenland et al, 2000; Stephens et al, 1995).

Only three out of fifteen studies failed to find any differences between exposed and unexposed populations. All three studies examined agricultural workers and had a number of methodological weaknesses. Both Ames et al (1995) and Rodnitzky et al (1975) failed to provide adequate information about exposure history making it impossible to determine whether the findings relate to short or long-term exposure to OPs. Neither provide any information about the work undertaken by their subjects (e.g. spraying, dipping, ground application); and both involve small sample sizes. Rodnitzky et al's (1975) study was limited further by the inclusion of individuals with a history of exposure to pesticides in the control group. The third study to report negative findings was by Jamal et al (2001) who grouped subjects according to whether they had peripheral nerve damage and then looked for corresponding evidence of central nervous system damage (i.e. cognitive impairment), which they did not find. Exposure history was not specified or used as a variable in this study. The overall value of these three studies is limited by major methodological weaknesses.

3.3.3.7.1 Potentially critical exposure variables

Studies which found subtle neurobehavioural deficits following exposure were of pest control operators (Steenland et al, 2000) and fruit tree farmers (Fiedler et al, 1997; London et al, 1997; Stephens et al, 2004). All studies included adequate outcome measures, although London et al (1997) had to modify their measures because of cross cultural issues. The study of pest control operators by Steenland et al (2000) involved study participants who had a relatively short history of exposure to OPs (average of 2.4 years) and this may account for the minimal findings. Studies by Stephens et al (2004) and Fiedler et al (1997) involved small sample sizes with limited power to detect associations, particularly small effect sizes. The study by London et al (1997) is particularly hard to interpret due to a number of methodological weaknesses including the inclusion of exposed persons in the control group and persons with a history of acute exposure in the exposed groups. It is possible that the exposure history of fruit tree farmers and pest control operators differs in some important way from other types of

agricultural work (e.g. sheep dipping or greenhouse work) or the manufacture of OPs, but more detailed information about the working practices of these different occupational groups would be required to determine if this is the case and could account for the different findings.

Remaining studies indicate that both intensity and/or duration of exposure may be important variables underlying the development of neurobehavioural problems. Studies by Srivastava et al (2000) and Amr et al (1997) of chemical plant manufacturers and Farahat et al (2003) of Egyptian pesticide applicators describe their study participants as having fairly prolonged, continuous, daily exposure to OPs as opposed to brief seasonal exposures reported in other occupational groups. Formulators work 40 hour days, every day and Egyptian applicators work 120 days per year. This contrasts with sheep dippers who may only be exposed to OPs on four occasions a year. Srivastava et al (2000), Amr et al (1997) and Farahat et al (2003) all found evidence of significant neurobehavioural problems following long-term exposure to OPs. Studies by Roldan Tapia et al (2005 and 2006) and Bazylewicz-Walczak et al (1999) of greenhouse workers found an association between cumulative exposure and neurobehavioural problems, particularly in those exposed for more than 10 years. The importance of 'prolonged exposure' was echoed by Mackenzie Ross et al (2007) who found an association between duration of exposure and impaired memory and motor function in a group of sheep dippers with an average of 14 years of exposure to OPs. All of these studies suggest neurobehavioural problems develop over several years and not after a single episode or season of exposure and that intensity and/or duration of exposure are critical causal factors.

With regard to the neurobehavioural domains affected, this review found considerable agreement between studies, for example, slowing of reaction times and impaired fine motor skills are almost universally found in all studies. Individuals who are more severely affected may show additional deficits in short-term memory and executive function. None of the studies reviewed report deficits in general intellectual functioning, semantic or autobiographical memory, perception or aphasias, agnosias or apraxias; and none report a positive association between cognitive function and exposure to OPs. Consistency of findings across many studies add strength to the hypothesis that exposure to OPs is linked to deficits in cognitive function and indicate that results are unlikely to be explained by random chance or bias.

3.3.3.7.2 How robust is this synthesis?

Clearly the individual studies described in this narrative review differ in terms of methodological quality and study populations and these factors may explain the variability in study findings. Although the majority of studies find an association between long term, low level exposure to OPs and impaired neurobehavioural function it is not clear which results are most reliable and should be used as the basis of policy decisions. It is important to get a clear answer to the question of whether low level exposure is harmful to human health, as many more individuals are likely to be at risk of long-term, low level exposure, rather than acute poisoning. Meta analysis is a useful method of summarising, integrating and quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. It combines information across studies thereby increasing the number of participants, reducing random error, narrowing confidence intervals and increasing statistical power to detect small effects that may be missed by individual studies which are too small to yield a valid conclusion (Zhou et al, 2002; Centre for Reviews and Dissemination (CRD), 2009). It represents each study's findings in the form of effect sizes. Combining the results of several studies in this way gives a more reliable estimate of whether a significant association exists between specified variables, than one study alone. Meta-analysis moves discussion away from individual studies towards an overview of a body of literature and it is considered to be the method of choice in situations where research findings may be used to inform public policy (CRD, 2009). The next chapter of this thesis reports the findings of a meta-analysis of the literature described in this chapter. As far as this author is aware, this will be the first systematic review of the literature to attempt quantitative evaluation of study findings using meta-analysis.

Chapter 4 : Neurobehavioural problems following low level exposure to organophosphate pesticides: A meta-analytic investigation.

4.1 Background

A meta-analytic investigation was undertaken to assimilate the data from the studies described in the previous chapter in order to determine the extent and nature of any association between exposure to OPs and cognitive impairment. Meta-analysis is a useful method of quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. It does this by representing each study's findings in the form of effect sizes which are a statistical standardisation of study findings based on standard deviation units. Combining information across studies in this way increases statistical power to detect small effects that may be missed by individual studies which are too small to yield a valid conclusion (Zhou, Obuchowski & Obuchowski, 2002).

4.2 Selection of studies

While the 15 epidemiological studies described in the previous chapter were identified as being suitable for inclusion in a meta-analysis, two of the identified studies (Jamal et al 2001; London et al 1997) failed to include sufficient data to calculate effects sizes such as sample sizes, means or standard deviations and had to be excluded. For example, London et al (1997) did not provide means and standard deviations for exposed and control subjects separately, but aggregated the data in their published paper. Jamal et al (2001) classified 74 UK sheep dippers into three groups according to whether they had signs of peripheral neuropathy, however exposure history was not specified or used as a variable in the analysis and data from appropriately matched controls was not provided. The overall study design is quite different from that involved in the other studies included in the review which undertook group contrasts involving exposed and unexposed populations. Jamal et al's study was therefore not considered comparable to the others included in the meta-analysis and was excluded from this review.

Three other studies either failed to report means and standard deviations for all of the group contrasts undertaken (Ames et al 1995; Rodnitzky et al 1975; Steenland et al) and

merely stated their findings were non significant, in which case an effect size of zero was assigned rather than omitting the study altogether, since this might have biased the results. However, it is important to note that this procedure leads to effect size estimates that are too small and is very conservative in nature (Rosenthal, 1995).

Finally, the study by Amr et al 1997 was included in only one part of the meta-anlaysis as it had a limited focus which was to determine the incidence of psychiatric disorder in pesticide applicators and formulators. Assessment of cognitive functioning was not undertaken. This left a final sample of 12 studies for inclusion in the first stage of the meta-analysis and these are summarised below (see Table 4.1). The aim of all of these studies was to determine the effect of long term, low level exposure to OPs on neurobehavioural function, but researchers examined a broad range of populations from chemical plant workers, pest control operatives, greenhouse workers, crop sprayers, sheep dippers and fruit tree sprayers. Studies were carried out on individuals from developed and developing nations and exposure history varied considerably from being continuous i.e. on a daily basis, to seasonal or infrequent (e.g. twice a year). Lifetime exposure also varied from an average of two to over twenty years.

4.3 Meta-analysis

The primary objective in undertaking a meta-analysis is to determine whether long-term, low level exposure to OPs is associated with neurobehavioural problems and if so, how strong the effect size is in terms of the mean effect size. A further research question is whether neuropsychological tests differ in their sensitivity to, or ability to identify nervous system effects of OP exposure in human populations.

Table 4.1 Table of studies included in the meta-analysis

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
MANUFACTURERS								
Srivastava et al 2000	Health risks associated with the manufacture of OP	59 Indian chemical plant workers 17 controls	Matched for age, sex. Controls better educated.	OP (quinalphos) & other	EHQ AChE	Average 6 yrs	Medical exam Digit Span Digit symbol Vigilance task	Similar AChE levels in both groups, but exposed had altered reflexes and neurobehavioural deficits, i.e. lower scores on digit span, digit symbol & vigilance task.
Amr et al 1997	Psychiatric morbidity amongst applicators & formulators	208 formulators 172 applicators 233 controls (mix of urban textile workers and rural residents)	Matched for age, socio-economic status, education.	OP, Organochlorines Carbamates, Pyrethroids	Years of exposure	Average 2 yrs	Psychiatric assessment - GHQ, DSM-IIIR	Frequency of psychiatric disorder (depression) higher amongst PF & PA than controls and those with longer duration of exposure (>20 years). Rates or reactive depression equivalent between groups, but rate of dysthymic not & higher than in general population.

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
PEST CONTROL								
Steenland et al 2000	Chronic neurological effects of OP exposure	191 current & former termiticide applicators 189 controls	Matched for age, sex. Controls better educated.	OP (Chlorpyrifos)	EHQ Urinary metabolites PON1	Average 2.4 yrs	Nerve conduction Clinical exam NES Battery: Finger tapping Hand-eye co-ord Reaction Time Continuous performance Symbol digit BVRT Pattern comparison Pattern memory Switching attention Digit span Serial digit Associate learning Associate recall Mood scales.	Exposed group reported more problems with memory, emotional state, fatigue and muscle strength but few differences noted on quantitative tests. Exposed were impaired on pegboard turning and some postural sway tests but were not significantly different from controls on other cognitive tests. 8 subjects who were acutely exposed had impaired reaction time and continuous performance.

OP organophosphate; NB neurobehavioural; PA pesticide applicator; PF pesticide formulator; AChE acetylcholinesterase; BuChE serum cholinesterase; PON1 paraoxonase 1; EHQ exposure history questionnaire; GHQ general health questionnaire; DSM-III diagnostic and statistical manual 3rd edition; AMIPB adult memory and information processing battery; HAD hospital anxiety and depression scale; WHO world health organisation NB core test battery; NES NB evaluation system battery.

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
FRUIT TREE								
Stephens & Sreenivasan 2004	Effect of long-term, low level exposure to OPs on NB function	37 orchard sprayers 26 pig farmers 31 construction workers	Matched for age & education	OP (chlorpyrifos)	EHQ	Average 14 yrs	NB battery same as 1995 study.	Orchard workers slower on syntactic reasoning than controls but no relationship with exposure index.
Fiedler et al 1997	Effect of long-term, low level exposure to OPs on NB function	27 US Fruit Farmers 42 cranberry & blueberry farmers and hardware store controls	Matched for age. Controls more educated.	OP no further data	EHQ	Average 27 yrs	Medical Exam Reaction time Stroop Pegboard Eye/hand co-ord Trails Digit span Digit symbol CVLT Visual reproduction Continuous visual memory test Information Naming Token Test MMPI-2	Exposed and controls had different reading scores and levels of education, so reading score was used as a covariate in the analyses. Fruit farmers have slower simple RT than controls. Fruit farmers split into high vs low exposure and groups differ in simple RT. No other differences found or alterations in mood/personality

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
GREENHOUSE WORKERS (GHW)								
Roldan-Tapia et al 2005	Continuous exposure to OPs (subsymtomatic) and NB effects	40 Spanish GHW 26 matched controls	Matched for age & education.	OP & Carbamates	BuChE EHQ	Average 11 yrs	Medical exam WHO core battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	Association between cumulative exposure and lower performance on verbal memory, visual memory and increased anxiety. Those exposed for more than 10 years also have lower scores on tests of visuo-spatial ability.
Roldan-Tapia et al 2006	Association between different levels of exposure to OPs & NB function	24 Spanish GHW with a history of acute exposure. 40 workers with low level exposure (high vs low groups) 26 controls	Matched for age & education, but low level exposure group younger than other two groups.	OP (metamidophos, fenamiphos, malathion, fosetyl, dimethoate) & Carbamates	BuChE EHQ	Average 11 yrs	Medical exam WHO core battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	Subjects had reduced visuo-motor, perceptual & constructive abilities, verbal learning, speed of processing and increased anxiety. Acutely exposed and those exposed for > 10yrs had similar profile of deficits. Those exposed for <10yrs and controls had similar profiles.

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
Bazylewicz-Walczak et al 1999	Behavioural effects of chronic exposure to OPs	51 Polish GHW (female) 25 controls (admin, canteen workers) matched for age,educ	Matched for age, education, alcohol use & smoking.	OP (dichlorvos, methamidophos, methidathion, pirimiphos-methyl) Carbamates Pyrethroids Dithiocarbamates	Air sampling Concentration on clothes	Average 12 yrs	WHO battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	No change in performance on NB tests pre/post season, but differences between controls and exposure groups on both occasions suggesting lifetime cumulative exposure affects NB function, but not a single episode of exposure.

OP organophosphate; NB neurobehavioural; PA pesticide applicator; PF pesticide formulator; AChE acetylcholinesterase; BuChE serum cholinesterase; PON1 paraoxonase 1; EHQ exposure history questionnaire; GHQ general health questionnaire; DSM-III diagnostic and statistical manual 3rd edition; AMIPB adult memory and information processing battery; HAD hospital anxiety and depression scale; WHO world health organisation NB core test battery; NES NB evaluation system battery.

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
PESTICIDE APPLICATORS								
Rodnitzky et al 1975	NB changes following chronic exposure to OPs	23 farm workers (12 farmers & 11 PA) 23 farmers not exposed in last 2 weeks, but may have a history of exposure during lifetime.	Matched for age & education. Controls have a history of exposure.	OP no further data	AChE. Exposed in last 2 weeks	Not reported	Verbal recall RT/vigilance task Choice reaction time Sentence repetition Proprioception	No significant differences between groups on cognitive tests but applicators had higher rates of anxiety than controls. AChE within normal limits but plasma levels slightly lower in PA than controls.
Ames et al 1995	Long-term, low level exposure to OPs and NB function. Does prevention of acute poisoning prevent chronic ill health	45 US (incl Hispanic) PA with history of AChE depression 90 controls (friends)	Controls younger and more educated	pesticides in general - no other data	Records - looking for cholinesterase inhibition without symptoms	Not reported	Nerve conduction Finger tapping Sustained attention Eye-hand co-ord Reaction time Digit symbol Digit span Pattern memory Santa Ana dexterity Pursuit aiming	No group differences. Preventing acute poisoning prevents chronic sequelae

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
Farahat et al 2005	NB effects of pesticide exposure	52 Egyptian PA 50 controls (admin clerks)	Matched for age, sex, education	OP (chlorpyrifos, Dusban, Curacran, Hostathion, Thimet, Profenofos, Triaziphos, Phorate), Carbamates, Pyrethroids	AChE EHQ	Average 18 yrs	Medical Exam Similarities Digit symbol Trails Block Design PASAT Letter cancel Digit span Benton visual form discrimination test Story recall EPQ	PA obtained lower scores on similarities, tests of attention, visual memory and timed tests than controls and this did not correlate with AChE levels (so not due to current exposure) but did correlate with lifetime exposure. Neuroticism higher and symptoms of numbness & dizziness.

OP organophosphate; NB neurobehavioural; PA pesticide applicator; PF pesticide formulator; AChE acetylcholinesterase; BuChE serum cholinesterase; PON1 paraoxonase 1; EHQ exposure history questionnaire; GHQ general health questionnaire; DSM-III diagnostic and statistical manual 3rd edition; AMIPB adult memory and information processing battery; HAD hospital anxiety and depression scale; WHO world health organisation NB core test battery; NES NB evaluation system battery.

Study	Research question	Participants	Controls matched	OP compounds	Exposure Measures	Years of exposure	Measures	Results
SHEEP DIPPERS								
Stephens et al 1995	Repeated, long term exposure to OPs & NB function	146 sheep farmers 143 controls (quarry workers)	Farmers older and more educated. Controls consume more alcohol.	OP (diazinon, chlorfenvinphos, propetamphos)	EHQ	Average 15 yrs	Digit span Visual memory Reaction time Digit symbol Syntactic reasoning Word learning Category search	Farmers slower than controls on all timed tests, impaired attention but memory intact. Split into 5 levels of exposure groups and highest exposure group worst on syntactic reasoning (even after controlling for covariates) Greater vulnerability to psychiatric disorder.
Mackenzie Ross et al 2007	Nature & extent of NB problems in farmers who report chronic ill health.	25 sheep dippers 22 controls	Matched for age, sex, years in education.	OP (diazinon, chlorfenvinphos, propetamphos)	EHQ	Average 14 yrs	WAIS-R AMIPB Trails A&B Face recognition Line orientation verbal fluency NART Stroop HAD (mood)	Exposed had lower scores on tests of mental flexibility, verbal memory and 70% had mood disorder. Many reported 'dippers flu' which may be indicative of unrecognised acute toxicity.

OP organophosphate; NB neurobehavioural; PA pesticide applicator; PF pesticide formulator; AChE acetylcholinesterase; BuChE serum cholinesterase; PON1 paraoxonase 1; EHQ exposure history questionnaire; GHQ general health questionnaire; DSM-III diagnostic and statistical manual 3rd edition; AMIPB adult memory and information processing battery; HAD hospital anxiety and depression scale; WHO world health organisation NB core test battery; NES NB evaluation system battery.

4.3.1 Calculation of effect sizes and effect size formulas

Many measures of effect size have been proposed and the most common are Pearson's correlation coefficient, r, Cohen's d (and its multiple variants such as Hedges' g, Glass's Delta etc), and the odds ratio (OR) (Field & Gillett, 2010). Since all of the papers selected for meta-analysis involve group contrasts, Cohen's d seems the most appropriate formula for the current meta-analysis as it is based on the standardised difference between two means. It is calculated by subtracting the mean of one group from the mean of another and standardising it by dividing by the population standard deviation.

$$d = \frac{M_1 - M_2}{\sigma}$$

However, several different methods of estimating the population standard deviation from sample data exist, such as using the root mean square standard deviation, the pooled standard deviation or the control group standard deviation. Previous research suggests that exposure to OPs may have differential effects on different individuals and therefore exposure will not only affect the mean of any outcome variables used in a study but also the variance. In such cases it is best to estimate the ES using only the standard deviation of the control group because it is a better estimate of the population variance (Lipsey & Wilson, 2001). Glass's Delta is a variation of Cohen's d which uses the standard deviation for the control group when calculating effect sizes:

$$\Delta = \frac{M_1 - M_2}{\sigma_{control}}$$

Effect sizes are usually calculated using population means but in this study effect sizes were also calculated using population medians to ensure that any significant effect sizes identified in the analyses were not due to outliers. In this case, effect sizes were calculated using the two abovementioned formulae but substituting mean scores for medians. The median (middle value) is used as a measure of central tendency when a data distribution is skewed because it is considered to be a better indicator of the central location of data than the arithmetic mean, when the data contains outliers. However, a potential disadvantage of using the median occurs when there are scores of zero in a

data set, as the median value can become ‘swamped’ by them and is likely to be lower and more conservative than the arithmetic mean.

The meta- analysis was performed in several stages. Firstly (step 1), multiple effect sizes were calculated for each study incorporating data from all of the psychometric tests administered in a given study, but omitting the data from mood questionnaires as the latter is based on subjective self report rather than objective measures of cognition. However, results could be biased by a small number of studies producing multiple effect sizes, so an overall effect size was calculated per study so that each study contributed a single effect size. Thus a single mean effect size within each study was computed before undertaking the meta-analysis across studies. Four methods of calculating effect size were used (Cohen’s d utilising mean scores; Cohen’s d utilising median scores; Glass’s delta utilising mean scores; and Glass’s delta utilising median scores). Analyses were undertaken to establish whether any particular method used to calculate effect sizes alters the overall findings. The second stage of analysis (step 2) involves examination of effect sizes found in different studies and establishing the variance of effect size distributions (heterogeneity) to determine whether studies are comparable. Finally the influence of potential moderator variables on the overall findings was considered such as task parameters (outcome measures) and population characteristics (of both exposed and control samples).

4.3.2 Method of meta-analysis

All analyses were conducted using custom-written syntax for SPSS. The meta-analysis was computed by the Mix 1.7 programme and a random effects model was used as it is assumed that there will be random differences between studies which are not solely due to sampling error, but are associated with variations in procedures. Random effects models are generally considered to be more appropriate than fixed effects models when analysing behavioural, social and health science data (Field & Gillett, 2010).

Step 1 – establishing whether the method used to calculate effect sizes affects the overall findings.

Table 4.2 shows the included studies, overall single effect sizes for each study based on the mean and median. Effect size calculations using both Cohen’s d and Glass’s formula are reported and the number of psychometric tests administered in each study upon

which the ES calculations were based appear in the final column. Findings appear similar regardless of whether Cohen's d or Glass's Delta formula are used, and regardless of whether effect size calculations are based on population means or medians, but meta-analysis were undertaken on all of the different effect size calculations to determine whether any particular formula produces different findings. All 12 studies were entered into the meta-analysis and all four formulae for calculating effect sizes were used. Meta-analysis revealed similar findings regardless of whether Cohen's d or Glass's Delta formula were used, and regardless of whether effect size calculations were based on population means or medians (see Tables 4.2 and 4.3).

All future analyses were undertaken using Glass's Delta, based on population means to allow all of the data to be incorporated. The rationale for this is as follows. As previously explained in Section 4.3.1 the meta-analysis was performed in several stages. Firstly, multiple effect sizes were calculated for each study incorporating data from all of the psychometric tests administered in a given study. Secondly, an overall effect size was calculated per study. As the initial data sets upon which an overall effect size was calculated for each study contained a number of zero effect sizes, median scores were deemed less appropriate for use in this meta-analysis. Glass's delta seems the more appropriate measure for neurotoxicological research because it is important to take account of the possibility that exposure to OPs may have differential effects on different individuals and is likely to impact on variance. In such cases it is better to estimate the ES using only the standard deviation of the control group.

Table 4.2 Effect size calculations using Cohen's d and Glass Delta formulas, based on population means and medians.

Author	Ref	Study date	Participants	Sample size; Exposed vs controls	Cohen's d Median	Cohen's d Mean	Glass Delta Median	Glass Delta Mean	Standard error	No of ES per study
Rodnitzky	6	1975	US farmers And PA	46 23/23	0.00	0.00451	0.00	-0.00437	0.295	15
Ames	3	1995	US PA	135 45/90	0.00	0.0516	0.00	0.0515	0.183	7
Stephens	4	1995	UK sheep dippers	289 146/143	-0.0148	-0.0956	0.0154	-0.135	0.118	13
Fiedler	12	1997	US Fruit Tree Sprayers	99 57/42	-0.0997	-0.0969	-0.103	-0.0975	0.203	18
Bazylewicz-Walczak	9	1999	Polish GH workers	51 26/25	-0.338	-0.282	-0.364	-0.282	0.280	22
Srivastava	1	2000	Indian manufacturers	76 59/17	-0.921	-1.007	-0.869	-1.0119	0.275	3
Steenland	13	2000	Termiticide PA	380 191/189	-0.00	-0.0327	0.00	-0.0327	0.103	11

Farahat	2	2003	Egyptian PA	102 52/50	-0.548	-0.536	-0.553	-0.541	0.198	12
Stephens	8	2004	UK FT sprayers	68 37/31	-0.126	-0.0344	-0.118	-0.0538	0.243	11
Roldan-Tapia	11	2005	Spanish GH workers	66 40/26	0.0289	0.0102	-0.0294	0.0435	0.252	21
Roldan-Tapia	10	2006	Spanish GH workers	46 20/26	-0.0747	-0.0167	0.0753	-0.0296	0.297	42
Mackenzie Ross	5	2007	UK sheep dippers	47 25/22	-1.264	-1.198	-1.530	-1.617	0.292	21

PA = pesticide applicators; GH = greenhouse workers; FT = fruit tree sprayers

Table 4.3 Meta-analysis using a random effects model incorporating 12 studies and 1,405 participants and involving four different methods of calculating effect size.

	Cohen's d Mean	Cohen's d Median	Glass Delta Mean	Glass Delta Median
Overall ES	-0.236	-0.237	-0.279	-0.259
95% CI lower	-0.429	-0.446	-0.506	-0.486
95% CI upper	-0.042	-0.027	-0.052	-0.031
z	2.386*	2.217*	2.413*	2.227*
t^2	0.673	0.086	0.109	0.110

-Asterisks denote significant effects: * p<.05

Step 2 – analysis of the findings by study- Glass's Delta, based on the population mean is reported.

Various graphical techniques exist to illustrate the central tendency, variability and normality of effect size distributions and the stem and leaf, forest and funnel plots are particularly popular. Figure 4.1 is a stem and leaf plot depicting the effect sizes (based on Glass's delta and the population mean) for each of the 12 studies included in the meta-analyses. Figure 4.2 is a forest plot depicting the effect sizes, 95% confidence intervals and the amount of variation between studies.

Stem	Leaf
1	
0	0,0
-0	0,0,0,0,1,1,3,5,
-1	0,6

Figure 4.1 Stem and leaf display of effect size data from 12 studies investigating the effect of low level exposure to OPs on neuropsychological functioning.

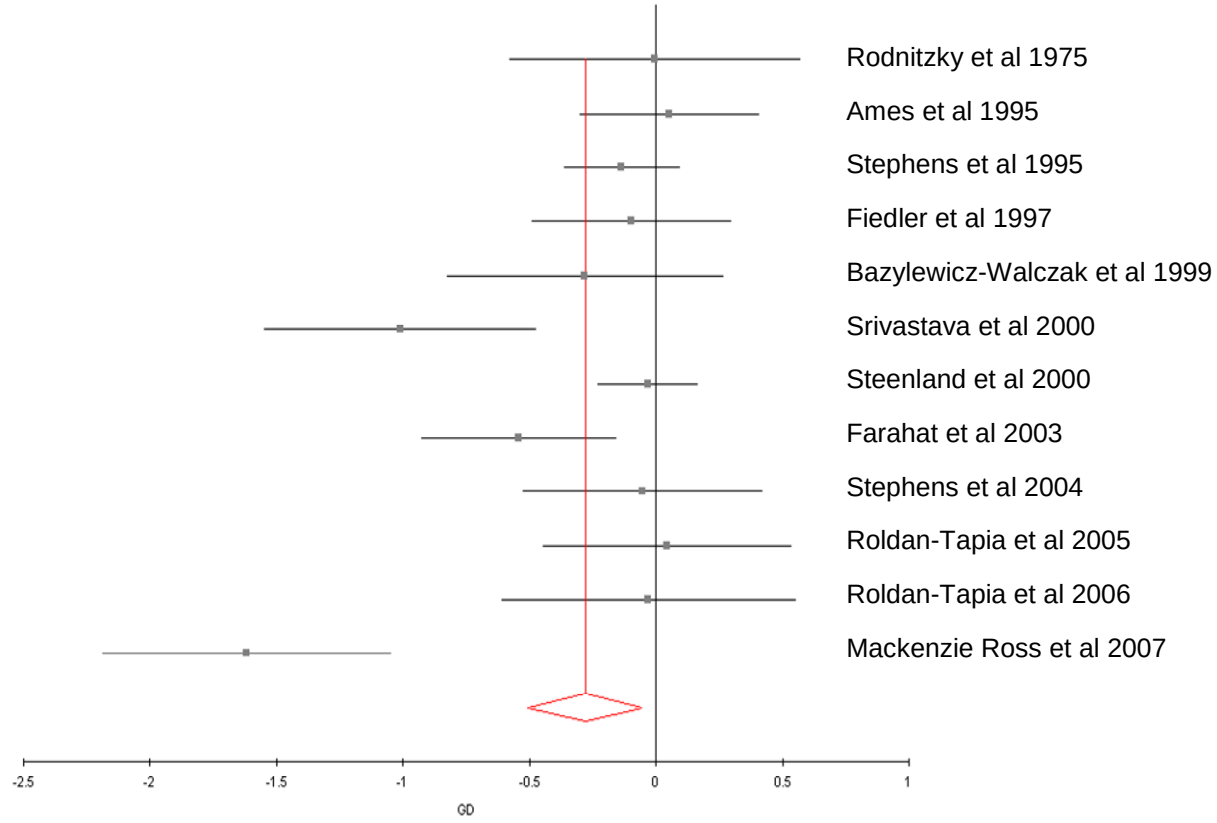


Figure 4.2 Forest plot depicting overall effect sizes (based on Glass's delta) derived from 12 studies in date order and corresponding 95% confidence intervals.

The first thing to note is the direction of the effect sizes. Of the12 studies, ten showed a negative effect, and two showed a positive effect. If no consistent pattern existed then one would expect to see a random pattern of effect sizes scattered in both directions at a 50:50 ratio. A 2-tailed binomial test (with .5 set as the test proportion) revealed that the proportion of negative effects sizes seen in these studies were significantly higher than expected (p=.04). This predominantly negative pattern indicates poorer performance in exposed workers than unexposed controls. There were only two exceptions to this. Firstly, Ames et al (1995) failed to report necessary statistical parameters for the majority of the psychometric tests in the study. In these cases effect sizes of zero were assigned before undertaking the meta-analysis. This is a conservative approach which is likely to have lowered the overall effect size for this study. Secondly, Roldan-Tapia et al (2005) failed to find significant differences in performance between exposed and unexposed populations on the vast majority, but by no means all, of the tests included in their assessment battery. Most of the effect sizes illustrated in Figure 4.2 cluster around-.03 (overall ES -0.279, p=0.0158) but there is some variation in effect sizes ($t^{\wedge 2}$ 0.1089)

with studies by Srivastava et al (2000) and Mackenzie Ross el al (2007) showing the largest effect sizes.

Srivastava et al (2000) examined 59 Indian workers recently exposed to different chemicals during the manufacture of 'quinalphos' and 17 control subjects. 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 nervous system damage. However, controls were not matched to the exposed group for level of education and would be expected to outperform the exposed cohort and this may explain why the effect size produced by this study was larger than that observed in other studies. Having said that, a number of other studies have utilised unmatched control groups (Ames et al, 1995; Fiedler et al, 1997; Steenland et al 2000; Stephens et al, 1995) and the first two of these produced low or zero effect sizes; so an alternative explanation might be that Srivastava et al's study participants had more prolonged exposure than other groups as they were involved in the manufacture of OPs on a daily basis rather than the occasional, seasonal application of OPs.

Mackenzie Ross et al (2007) compared 25 farm workers with a history of apparent low level exposure to sheep dip with 22 non-exposed healthy volunteers on neuropsychological tests. Farm workers performed significantly worse than non-exposed healthy volunteers on tests of mental flexibility, response speed and memory; and over 70% suffered from mood disorder. However, the sample size was small and self selected making it unclear how representative they are of the farming community as a whole. The sample is different from others reported in the literature in that a large proportion of study participants had retired on ill health grounds, whereas other studies recruited participants who were still fit enough to be in employment. Furthermore, participants in the Mackenzie Ross study were involved in litigation and so there are a number of factors such as potential secondary gain or the possibility that participants constitute a subgroup of people who are particularly vulnerable to the effects of OPs, which could explain the large effect size produced by this study.

In order to determine whether the Mackenzie Ross et al study, which produced the largest effect size, was biasing the findings, analyses were repeated excluding this study. It is possible to statistically test for homogeneity to determine whether effect

sizes from different studies show more variation than would be expected from sampling error alone and gives an indication of whether studies are broadly comparable (Lipsey and Wilson, 2001). Random effects meta-analyses provide a measure of absolute variance reported as $t^{\wedge 2}$. If it is near to zero then any dispersion in effect sizes is due to random error. When $t^{\wedge 2}$ moves away from zero it suggests some of the variance is real and due to fundamental methodological differences between studies.

Table 4.4 Meta-analysis using a random effects model illustrating the effect of excluding the study by Mackenzie Ross et al.

	Glass Delta Mean *Mackenzie Ross et al study excluded*	Glass Delta Mean Mackenzie Ross et al study included
Overall ES	-0.1656	-0.279
95% CI lower	-0.3232	-0.5056
95% CI upper	-0.008	-0.0524
z	2.0601*	2.4129*
$t^{\wedge 2}$	0.0278	0.1089

-Asterisks denote significant effects: * p<.05

Excluding the study by Mackenzie Ross et al does not render the overall findings non significant, but does result in a large reduction in the heterogeneity rating. Removal of this study alters the overall balance and comparability of remaining studies which appear more homogeneous once it has been excluded; but the overall effect size produced by the meta-analysis remains significant. The convention with regard to interpreting effect sizes is that d=0.2 to 0.5 is 'small'; 0.5-0.8 is medium and >0.8 is large; hence the overall effect size found in the current analyses of between -0.1656 and -0.279 (depending upon whether the study by Mackenzie Ross is included or not) can be classified as small.

4.3.3 Influence of study publication date

Another interesting observation from the forest plot depicted in Figure 4.2 is the fact that nine out of ten studies published after 1995 found negative effect sizes between -

0.03 and -1.62, the only exception being a study by Roldan Tapia et al in 2005 which produced a positive effect size of 0.04. The earlier studies by Ames et al (1995) and Rodnitzky et al (1975) which produced the lowest effect sizes were beset by methodological weaknesses which might account for their findings. For example Ames et al (1995) examined US farm workers, some of who had a history of cholinesterase inhibition and some who did not. None had a history of frank poisoning. No group differences were found and the authors conclude that prevention of acute poisoning prevents development of chronic neurobehavioural impairment. Unfortunately, Ames et al failed to provide any information about exposure history for their cohort and did not report means and standard deviations for all of the group contrasts undertaken. They merely stated their findings were non-significant which meant an effect size of zero had to be assigned to a number of group contrasts for meta-analytic purposes which is very conservative. Rodnitzky et al (1975) examined 23 farmers / commercial pesticide applicators in Iowa who had used OP compounds within 2 weeks of testing, but were asymptomatic and compared them with 23 non-exposed farmers. Groups did not differ significantly on tests of memory or RT. However, Rodnitzky et al (1975) also failed to provide adequate information about exposure history making it impossible to determine whether the findings relate to short or long-term exposure to OPs. Neither Ames et al or Rodnitzky et al provide any information about the work undertaken by their subjects (e.g. spraying, dipping, ground application); and both involve small sample sizes. Rodnitzky et al's (1975) study was limited further by the inclusion of individuals with a history of exposure to pesticides in the control group, which means the two groups would not be expected to differ significantly on tests of neurobehavioural functioning.

The only other study which produced an overall positive effect size was Roldan-Tapia et al 2005. They 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). More than twenty tests of neurobehavioural functioning were included in the assessment battery, but exposed and control subjects obtained similar scores on the vast majority of tests. This may be why overall, a negative effect size was not apparent. However, participants with a history of exposure to OPs, particularly of more than 10 years duration, were found to obtain lower scores on tests of delayed verbal memory, visual memory, integrative perception and visuo-constructional praxis

and measures of anxiety and the authors conclude that long-term exposure to pesticides can cause specific neurobehavioural problems.

4.3.4 Does the type of control group affect the strength of the ES.

The majority of studies included in this meta-analysis matched their exposed and unexposed groups on important variables which are known to influence performance on neuropsychological tests such as age, gender and years of education. The exceptions being Ames et al (1995), Srivastava et al (2000) and Fiedler et al (1997) who utilised control groups with a greater degree of education than the exposed group. This may have biased their results since differences between the groups may be due to pre-existing differences in premorbid IQ rather than exposure history. In terms of how this might affect the results of the current meta-analysis, an effect size of zero was assigned to a number of group contrasts in the study by Ames et al for the reasons cited earlier which means the results of the meta-analysis will not have been affected by the fact that their exposed and unexposed cohorts were not matched. However, the effect sizes produced by Srivastava et al and Fiedler et al may be inflated by the fact that their exposed and unexposed cohorts were not matched in terms of education. Fiedler et al went on to explore the amount of variance in reaction time which was due to the confounding effects of education and age by undertaking regression analyses. They found that the exposed cohort had significantly slower reaction times (dominant hand) than the controls even after controlling for the influence of age and education on neuropsychological function.

4.3.5 File drawer analysis

One potential bias in meta-analysis arises from the fact that significant findings are more likely to be published than non significant findings and this is known as publication bias or the' file drawer problem' (Field & Gillett 2010; Rosenthal, 1979).

The funnel plot is a graphical technique for exploring publication bias. It displays effect sizes plotted against the inverse standard error. An unbiased population would show a cloud of data points that is symmetric around the population effect size and should have the shape of a funnel. An asymmetric funnel suggests either publication bias, a difference between smaller and larger studies or an inappropriate effect measure (Field & Gillett 2010). Figure 4.3 displays a funnel of effect sizes from the current analysis.

The vertical axis displays the inverse standard error, the horizontal axis the effect size and the line in the middle is the pooled estimate from the meta-analysis. Most of the studies included in the analysis produce effect sizes which are close to the pooled estimate and the funnel is relatively symmetrical. The exception being the two studies which produced effect sizes to the left of the common effect and outside of the funnel. These are the studies by Mackenzie Ross et al (2007) and Srivastava et al (2000) described earlier which were performed on particularly high risk populations which produced unusually large effect sizes.

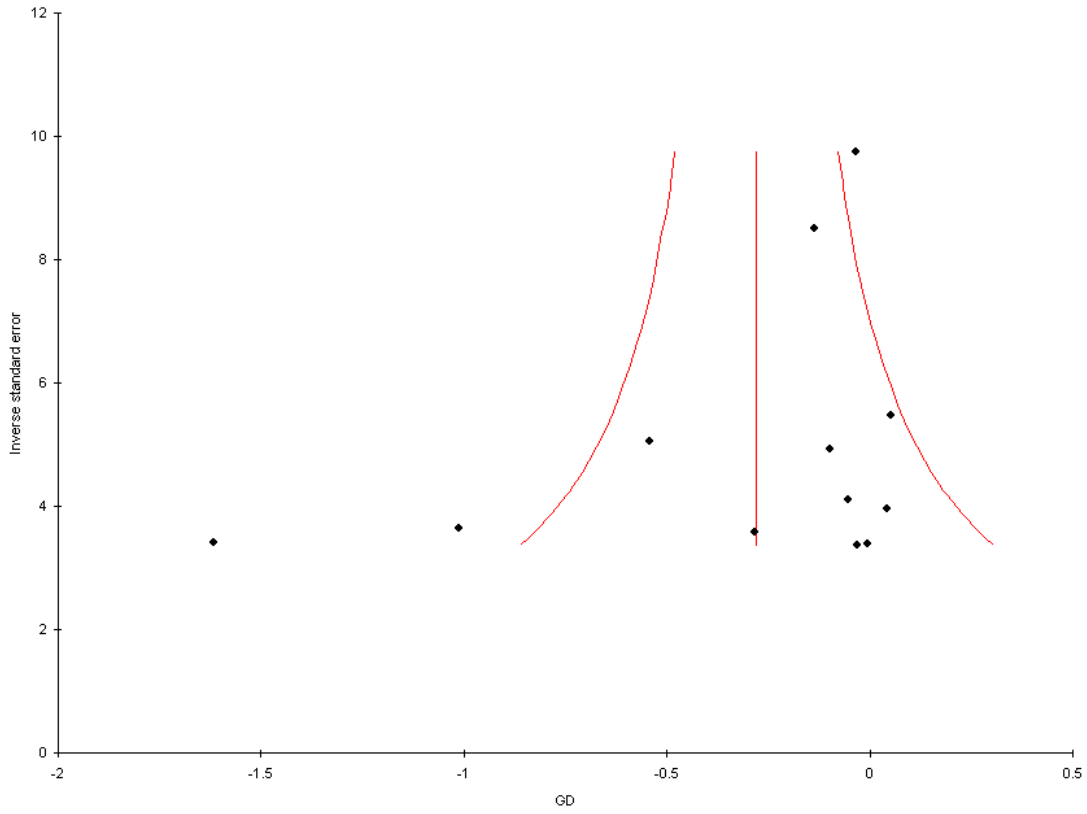


Figure 4.3 Funnel plot displaying effect sizes plotted against the inverse standard error.

Other methods of exploring publication bias that have been developed since the funnel plot can be difficult to interpret as funnel asymmetry may be due to publication bias, clinical or methodological heterogeneity between studies (Lipsey & Wilson 2001). In order to address the specific concern that studies with negative findings may not be published Rosenthal (1979) developed a statistic known as the fail safe N which estimates the number of unpublished studies reporting null results that would need to exist to turn a significant population effect size estimate into a non-significant one (Field & Gillett 2010). The fail safe N formula is:

$$K0 = \frac{k\ ESk\ -1}{Esc}$$

The number of studies with a zero effect needed to make the results of the current meta-analysis non significant would be 45. Thus publication bias is not a significant concern.

4.3.6 Effect of cognitive task

Neuropsychological tests are useful tools for exploring the early effects from exposure to toxic substances (Lezak, 1984; Lucchini et al, 2005) but tests vary in terms of their sensitivity to neurotoxic effects and clinical utility for toxicity diagnoses (Hartman, 1995). Some cognitive functions appear to be affected to a greater degree than others by exposure to OPs and tests of psychomotor speed, reaction time, fine motor control, attention and memory are particularly sensitive to OP exposure. Non verbal abilities tend to be affected to a greater degree than verbal abilities although why this should be the case is poorly understood (Anger, Otto & Letz, 1996; Anger et al, 1997; Anger et al, 2000; Hartman 1995; Lucchini et al 2005). In contrast tests of vocabulary and general knowledge do not appear sensitive to neurotoxic effects, but are often included in assessment batteries as estimates of premorbid ability.

The current meta-analysis incorporated data from all of the psychometric tests administered in a given study (i.e. multiple effect sizes were calculated) and then a single mean effect size within each study was computed before undertaking the meta-analysis. To determine whether task parameters might influence effect sizes the meta-analysis was repeated but this time cognitive tests were grouped into cognitive domains and a single effect size was calculated for each domain by averaging the effect sizes across all measures within that domain. Table 4.5 summarises the results of meta-analysis by cognitive domain. For each domain, the first row illustrates the effect sizes produced by all studies whilst the second row illustrates the findings when the study by Mackenzie Ross et al (2007) which produces the largest effect sizes, is removed.

Table 4.5 Meta- analyses by cognitive domain (italics illustrate the findings when the study by Mackenzie Ross et al (2007) is removed).

Cognitive Domain	No studies	Overall ES	Lower CI	Upper CI	z	t^2
Working Memory	11	-0.311	-0.584	-0.037	2.224*	0.160
	10	*-0.226*	*-0.478*	*0.027*	*1.735*	*0.148*
Visual Memory	9	-.0206	-0.521	-0.012	0.041*	0.101
	8	*-0.163*	*-0.365*	*0.038*	*1.592*	*0.040*
Verbal Memory	8	-0.103	-0.467	0.261	0.555	0.225
	7	*-0.085*	*-0.071*	*0.242*	*1.070*	*0.006*
Attention	8	-0.307	-0.598	-0.016	2.070*	0.126
	7	*-0.174*	*-0.393*	*0.046*	*1.549*	*0.045*
Speed	12	-0.505	-0.905	-0.105	2.472*	0.445
	11	*-0.263*	*-0.472*	*-0.055*	*2.473**	*0.077*
Executive function	9	-0.331	-0.748	0.085	1.561	0.353
	8	*-0.105*	*-0.361*	*0.152*	*0.802*	*0.092*
Visuo-spatial	4	-0.504	-0.749	-0.26	4.042***	0
	3	*-0.452*	*-0.723*	*-0.182*	*3.278****	*0*
Language	6	-0.269	-0.619	0.081	1.507	0.134
	5	*-0.042*	*-0.198*	*0.114*	*0.529*	*0*
FMC	3	-0.177	-0.348	-0.007	2.036*	0
Mood	4	-0.308	-0.987	0.371	0.889	0.419

-Asterisks denote significant effects: * p<.05, ** p<.01 and ***p<.001

The neuropsychological tests which produced the largest effect sizes included tests of working memory (digit span), psychomotor speed, fine motor control and visuo-spatial ability. Figure 4.4 shows stem and leaf displays of effect size data for the cognitive domains which produced the largest effect sizes.

Psychomotor speed (11 studies)

Stem	Leaf
1	
0	0,0,0.0
-0	0,1,2,5,5,7,8
-1	

Working memory (10 studies)

Stem	Leaf
1	
0	0,0,0,1,1
-0	1,2,4,6
-1	3

Fine motor control (3 studies)

Stem	Leaf
1	
0	
-0	2,2,2
-1	

Visuo-spatial ability (3 studies)

Stem	Leaf
1	
0	
-0	4,4,5
-1	

Figure 4.4 Stem and leaf displays of the effect size data for the cognitive domains which produced the largest effect sizes (Mackenzie Ross et al study removed).

4.4 Discussion

The literature review described in Chapters 3 and 4 was carried out to investigate the functional consequences of long term low level exposure to OPs. Whilst evidence exists to support the view that high level/ acute exposure to OPs is detrimental to human health, the possibility that long-term low-level exposure to OPs causes ill health is controversial. Previous research has produced inconsistent findings, possibly because a large body of literature exists concerning the neurotoxicity of OPs incorporating different methodologies, populations examined and outcome measures. Meta-analysis is a useful method of quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. However, the results are only meaningful if the studies included and aggregated deal with similar constructs and relationships and utilise similar statistical analyses. Therefore strict inclusion and exclusion criteria were applied to the literature review to limit it to relevant, high quality studies of human adult populations which utilise similar

methodologies. This was done to ensure studies included in the review were comparable (homogeneous).

Although more than 500 published papers were identified concerning the impact on health of exposure to OPs, 483 were excluded as they did not address low level exposure to OPs and neurobehavioural functioning in adult populations. After removing articles that had been duplicated by different search strategies, failed to meet exclusion and inclusion criteria or failed to provide relevant statistical information required for meta-analysis, a final sample of 13 studies were identified as suitable for inclusion in this review. The majority of studies were of individuals who had been exposed to a mixture of pesticides, OPs being just one of the chemicals involved. All studies involved comparisons of exposed and unexposed individuals and provided quantitative measures of exposure and neurobehavioural outcomes.

Meta-analysis assimilated the data from these studies in order to determine the extent and nature of any association between exposure to OPs and cognitive impairment. Data from more than 1,400 participants was aggregated in order to produce a more reliable estimate of the association between exposure to OPs and neuropsychological impairment. The analyses show that overall a significant association exists between exposure to low levels of OPs and decrements in cognitive function which is small in magnitude. Working memory, psychomotor speed, fine motor control and visuo-spatial ability were affected to a greater degree than other cognitive domains such as language and general knowledge.

Methodological differences between studies make it difficult to comment further on the precise nature of the relationship between exposure to OPs and neurobehavioural functioning. A number of important questions remain unanswered, for example, the critical exposure variable remains unclear; is it dose, intensity, frequency or duration of exposure? Is there a simple, continuous, linear dose-response relationship or a stepwise or curvilinear relationship. If the latter, can a threshold be identified which if reached trigger symptoms of ill health? Is the dose-response relationship mediated by other factors such as genetic differences between people in their capacity to detoxify chemicals or the synergistic effects of chemical combinations? What is the time course of development of neurobehavioural problems and can they be ameliorated? Have the human health risks of exposure been underestimated by previous studies, the majority of

which have been of individuals fit enough to be in employment and have not included individuals who have left the profession because of disabling disease. Have the human health risks of exposure been overestimated by previous studies because inappropriate or unmatched comparison groups have been used; or the potentially confounding effects of prior medical and psychiatric history have not been considered? Might the apparent association between exposure to OPs and diminished neurobehavioural function be due to factors other than exposure such as stressful life events, beliefs, attributions or personality characteristics?

4.4.1 What are the critical exposure variables?

Although the current review utilised strict inclusion and exclusion criteria to limit the analyses to studies incorporating similar methodologies, there remained a degree of heterogeneity amongst studies, most notably in terms of the populations examined. Different occupational groups were evaluated including chemical plant manufacturers, pest control operatives, greenhouse workers, fruit and crop sprayers and sheep dippers. These populations differ considerably in terms of intensity and frequency of exposure which can range from a couple of days a year to several months or even daily exposure in the case of manufacturers. Indeed, Srivastava et al (2000) and Amr et al (1997) who examined chemical plant manufacturers and Farahat et al (2003) who examined Egyptian pesticide applicators describe their study participants as having fairly prolonged, continuous, daily exposure to OPs as opposed to brief seasonal exposures reported in other occupational groups. Formulators work 40 hour days, every day and Egyptian Applicators work 120 days per year. This contrasts with sheep dippers who may only be exposed to OPs on as little as two to four occasions a year (Mackenzie Ross et al, 2007; Stephens et al 1995). The populations included in this review also differ in terms of their country of origin, some of the largest effect sizes being produced by studies from developing nations (Amr et al 1997; Farahat et al 2000; Srivastava et al 2000) where daily exposure is not only more frequent and intense; but heat and humidity may alter the characteristics and toxicity of chemical products and influence decisions regarding the use of personnel protective clothing. Linguistic differences and possible illiteracy may mean instructions for use, storage and other health and safety advice is not followed and economic factors may mean products that have been banned from other countries due to health and safety concerns may still be in use.

Lifetime cumulative exposure may also be an important variable underlying the development of neurobehavioural problems and this also ranged considerably between studies from as little as 2 years to over twenty years. Srivastava et al (2000), Amr et al (1997) and Farahat et al (2003) all found evidence of significant neurobehavioural problems following long-term exposure to OPs. Studies by Roldan Tapia et al (2005 and 2006) and Bazylewicz-Walczak et al (1999) of greenhouse workers found an association between cumulative exposure and neurobehavioural problems, particularly in those exposed for more than 10 years. The importance of 'prolonged exposure' was echoed by Mackenzie Ross et al (2007) who found an association between duration of exposure and impaired memory and motor function in a group of sheep dippers with an average of 14 years of exposure to OPs. All of these studies suggest neurobehavioural problems develop over many years and not after a single episode or season of exposure.

It is important that future researchers group and analyse studies by occupation and country of origin because exposure history varies greatly between different occupational groups and even between nations. A variety of factors influence the amount of exposure an individual worker might have including the nature of the work (spraying, dipping, ground application), hours, days, years spent working with pesticides; whether the worker is exposed to a single chemical or a mixture of chemicals, use of protective measures (whether machinery was used to apply the pesticides, whether workers were protected by being in sealed cabs or using respirators or other protective clothing) environmental differences in temperature, humidity etc. It is also important to note that important differences may exist even within occupational groups for example the exposure histories of farm workers/pesticide applicators in different regions of the USA vary considerably. Some regions employ migrant workers who live in camps adjacent to fields where chemicals have been sprayed. This is not the case in California which has strict regulations for the protection of farm workers and a surveillance programme for reporting pesticide related illness.

4.4.2 Have the human health risks of exposure to OPs been underestimated?

Another issue raised by this analysis is the possibility that the human health risks of exposure to OPs may have been underestimated by previous studies, because the majority have recruited individuals who are fit enough to be in employment and have not included individuals who have left the profession because of disabling disease. The largest effect size noted in this review was produced by the Mackenzie Ross et al (2007) study, in which two thirds of study participants had retired or reduced their workload on ill health grounds. This is the only study to have included individuals who are no longer fit enough to be in employment and this may explain why this study produced the largest effect sizes. Individuals who have retired on ill health grounds may constitute a sub-group of persons who are particularly vulnerable to the effects of OPs either because of their exposure history or genetic factors which may influence their capacity to detoxify chemicals. It is therefore important that future researchers take account of the 'healthy worker' effect and examine individuals who have retired on ill health grounds in addition to those who are still fit enough to be in employment. Measures of susceptibility or vulnerability to the neurotoxic effects of OPs should also be included in future studies. For example human serum paraoxonase (PON1) hydrolyzes and detoxifies a variety of OPs and previous research suggests PON1 status differs amongst individuals (Richter & Furlong, 1999; Richter, Jarvik & Furlong 2008; 2009; Roest et al, 2007). PON1 polymorphisms may render some people at greater risk of developing ill health following exposure to OPs than others (Cherry et al, 2002; Mackness et al, 2003) and this should be explored by future researchers.

4.4.3 Have the human health risks of exposure to OPs been overestimated?

It is also possible that the human health risks of exposure been overestimated by previous studies because study participants were unrepresentative or high risk groups were recruited (Mackenzie Ross et al, 2007) or inappropriate or unmatched comparison groups may have been used. Steenland et al (2000), Fiedler et al (1997) and Srivastava et al (2000) utilised comparison groups who were more educated than the exposed cohort and would therefore be expected to obtain higher scores on neuropsychological tests because of pre-existing differences in premorbid ability. Unless further analyses are undertaken to take account of this issue it is difficult to determine the degree to which exposure predicts performance.

4.4.4 Do other factors account for inferior performance on neuropsychological tests in OP exposed populations?

A final issue raised by this analysis is whether any other factor, apart from exposure to OPs, can account for the inferior performance on neuropsychological tests observed in individuals with a history of low level exposure to OPs. Earlier reviews have referred to inconsistencies in neurobehavioural outcomes between studies which undermine the link between exposure and effect and suggest other factors may account for neurobehavioural symptoms such as health beliefs and attributional error, somatising tendencies (Solomon et al, 2007), stress and mood disorder or confounding factors like medical and psychiatric history. However, this review found considerable agreement between studies in terms of the neurobehavioural domains affected. For example, slowing of reaction times and impaired fine motor skills are almost universally found in all studies. Individuals who are more severely affected may show additional deficits in short-term memory and executive function (Bazylewicz-Walczak et al, 1999; Farahat et al, 2003; Mackenzie Ross et al, 2007; Roldan-Tapia et al 2005 and 2006; Stephens et al, 1995; Srivastava et al, 2000). None of the studies reviewed report deficits in general intellectual functioning, semantic or autobiographical memory, perception or aphasias, agnosias or apraxias; and none report a positive association between cognitive function and exposure to OPs. Consistency of findings across many studies argues against the alternative explanations listed above as the latter would produce more variable symptom profiles.

4.4.5 Conclusion

In summary, the majority of well designed studies find a significant association between long term, low level exposure to OPs and impaired neurobehavioural function, which is consistent, small to moderate in magnitude and concerned primarily with neurobehavioural functions such as working memory, psychomotor speed, fine motor control and visuo-spatial ability. One potential bias in meta-analysis arises from the fact that significant findings are more likely to be published than non significant findings. This is likely to be less of a problem when it comes to research on pesticides as organophosphate pesticides are the most widely used insecticides in the world and prevent millions of people from starving to death and from disease. Studies which produce negative findings are of great interest and are likely to be published as they

imply that continued use of these pesticide is safe. Nevertheless, further analyses were undertaken during this review to explore the issue of publication bias and revealed that the number of unpublished studies reporting null results that would need to exist to make the results of the current meta-analysis non significant would be forty five. It is therefore unlikely that the association between exposure to OPs and decrements in neurobehavioural function is entirely due to publication bias.

However, a number of unresolved issues remain in the literature concerning the precise nature of the relationship between exposure to OPs and neurobehavioural function and the strength of the association (has it been under or over estimated). This should be the focus of future studies. The next three chapters of this thesis present the findings of a four year empirical study of neuropsychological functioning and mood state in UK sheep farmers, in which methodological weaknesses of earlier studies identified in this review were addressed in the study design. For example study participants who had retired on ill health grounds were included to take account of the 'healthy worker effect'; study participants with a history of acute poisoning and those with a psychiatric or medical history that might otherwise account for ill health were excluded; exposure history was examined in detail and objective, reliable, valid, neuropsychological tests were used which are known to be sensitive to neurotoxic effects; and genetic factors that may render some individuals more vulnerable to the effects of OPs than others were explored.

PART 3

Neuropsychological and psychiatric functioning in UK sheep farmers exposed to low levels of organophosphate pesticides.

An empirical study.

Chapter 5 : Empirical study methodology

5.1 Background to the study

Organophosphate pesticides (OPs) 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, but there is a lack of reliable data on the scale of the problem. The immediate effects of high-level exposure to OPs have been well documented and involve inhibition of the enzyme acetylcholinesterase, causing changes in peripheral, autonomic and central nervous system function (cholinergic crisis). However, the possibility that long-term low-level exposure to OPs in doses below that causing acute toxicity may cause ill health is controversial (COT Report, 1999). In 2000 the UK Government agreed to fund a programme of research to address this issue and the author was successful in securing funding to undertake a 4 year study to determine whether low level exposure to OPs is associated with impaired neuropsychological and psychiatric functioning. The occupational group examined in this study were sheep farmers, as organophosphate pesticides were used extensively in the dipping of sheep in the UK and farmers are generally considered to have relatively low-level exposure to OPs. Methodological weaknesses of earlier studies were addressed in the study design such as; detailed exposure assessment and exclusion of study participants with a history of acute poisoning that might otherwise account for ill health; inclusion of study participants who had retired on ill health grounds to take account of the 'healthy worker effect'; exclusion of study participants with a psychiatric or medical history that might otherwise account for ill health; use of objective, validated psychometric outcome measures; and exploration of factors that may render some individuals more vulnerable to the effects of OPs than others such as the capacity to metabolise OPs.

5.1.1 Study objectives

Objective 1: To establish whether farm workers with a history of low level exposure to OPs (insufficient to cause acute intoxication) show evidence of physical disease, cognitive impairment and / or mood disorder.

Objective 2: To determine the nature and severity of physical symptoms, neuropsychological abnormalities and psychiatric disorder in farm workers with a history of low level exposure to OPs.

Objective 3: To determine whether individuals who have retired on ill health grounds constitute a particular subgroup of individuals who are more susceptible to the effects of OPs than others.

Objective 4: To investigate whether background factors (e.g. exposure history or capacity to metabolise OPs) render some individuals more vulnerable to the effects of OPs than others.

5.1.2 Study hypotheses

1. Farm workers with a history of low level exposure to OPs (insufficient to cause acute intoxication) will show evidence of cognitive impairment, mood disorder and physical ill health.

2. Farm workers will show a similar pattern of cognitive and emotional deficits as that reported in earlier studies by Stephens et al, 1995 and Mackenzie Ross et al, 2007, such as impaired response speed, working and general memory, mental flexibility and higher rates of emotional distress. Deficits in perceptual, intellectual reasoning and general verbal abilities are not expected.

5.2 Method

5.2.1 Ethical approval

Ethical Approval for this study was granted by the joint University College London / University College London Hospital committee and written informed consent was obtained from all study participants (see Appendix 3).

5.2.2 Study design

This is a cross sectional, case control study in which performance on neuropsychological tests of working and retired farmers, exposed to OPs in the course of their work, was compared with non exposed, working and retired healthy controls.

5.2.3 Statistical power

Using prior research to establish the sample size needed to detect a relationship between neuropsychological functioning and exposure history is challenging, as previous studies have utilized an array of different methodological designs, populations, psychometric tests and control groups (see previous chapter for further discussion of this). As such, it might be prudent to consider previously established effect sizes for studies which have investigated comparable exposed populations and/or cognitive domains which have consistently been found to show impairment.

The meta-analysis in Chapter 4 found that response speed, working memory, visual-spatial abilities and fine motor control showed significant impairment in the exposed cohorts. Using the effect sizes established by the meta-analysis for these domains (range = 0.2 - 0.5), a power analysis was conducted to determine the necessary sample size to detect relationships of similar magnitudes. Calculations revealed that an n of between 64 and 394 per group would be needed to obtain statistical power at the recommended .80 level (Erdfelder, Faul & Buchner, 1996) for 2-tailed tests. More specifically, studies which have investigated performance on psychometric tests using the same occupational group as the current study (UK sheep farmers), have found moderate to large effect sizes (0 .6 -0 .9) between cognitive function and exposure history (Mackenzie Ross et al, 2007; Stephens, 1995). Using this information, power analyses indicates that a sample size of between 21 and 45 individuals per group would be required to have 80% power to detect a relationship of this magnitude between neuropsychological functioning and exposure history using 2-tailed tests.

5.2.4 Participants

5.2.4.1 Exposed cohort

Two groups of sheep farmers were recruited: 67 working sheep farmers and 60 sheep farmers who had retired on ill health grounds. Both groups had a history of low level exposure to OPs, insufficient to cause acute intoxication resulting in medical intervention. Prior acute exposure was assessed by interview at the recruitment stage and again during the clinical study. Participants were asked whether they had ever felt so unwell immediately after dipping that they sought medical advice/intervention within 48 hours. If they had, they were excluded from the study. Remaining participants were

asked if they had ever suffered symptoms of ‘dippers flu’ after dipping for which they had not sought medical intervention. Since the main aim of this study was to determine whether there is a relationship between low level exposure to OPs and cognitive impairment, it was important to exclude any participants with a medical or psychiatric condition which might otherwise account for any deficits identified during assessment (see Table 5.1 for inclusion/exclusion criteria).

Table 5.1 Inclusion and Exclusion Criteria.

	Exposed cohort	*Unexposed cohort*
Inclusion	Aged between 18-70 years old	Aged between 18-70 years old
	Living in the South West or North of England.	Has worked in a rural area in the South West or North of England
	For the retired cohort, they must have retired on ill health grounds NOT age or economic reasons.	For the retired cohort, they must have retired on ill health grounds NOT age or economic reasons.
	Exposure to organophosphate pesticides for a minimum of 5 years prior to 1991 (safety regulations were implemented in 1992).	No known exposure to organophosphate pesticides.
	NO history of acute intoxication requiring medical intervention.	
Exclusion	History of psychiatric problems prior to exposure, neurological or serious medical problems which might otherwise account for any cognitive or emotional problems identified in this study.	History of psychiatric problems, neurological or serious medical problems which might otherwise account for any cognitive or emotional problems identified in this study.
	Substance abuse (including alcohol).	Substance abuse (including alcohol).
	Those with a history of acute organophosphate intoxication.	Exposure to organophosphates.

5.2.4.2 Healthy controls

It is extremely difficult to find a group of farmers in the UK who do not have a history of exposure to OPs and so it was necessary to identify a different occupational group to act as controls. A number of occupational groups were considered, but a primary concern was to find an occupational group with sufficient numbers of individuals who have retired on ill health grounds, that could be easily identified and accessed. Other important criteria included:

- The control group should be matched to farmers in terms of characteristics which have been shown by previous research to affect cognitive function (the main outcome variable of this study), for example: age, gender, education level, premorbid IQ.
- The control group should not have a history of exposure to organophosphates.

Variables such as the exact nature of the work undertaken, location, lifestyle, attitudes, life experiences were considered to be less important since these variables have not been shown by research to significantly affect performance on psychometric tests.

Rural police workers who have never worked in the farming industry were recruited as controls. Rural police workers undertake both administration and outdoor work as do farmers and a major advantage of using the police as a control group is that an organisation exists which holds a database of 80,000 retired members of the police force. Furthermore, the police force is divided into local constabularies making it possible to recruit police workers from the same geographical regions as sheep farmers.

None of the police workers included in the study had a history of exposure to OPs. 38 rural police workers who were fit enough to be in employment and 40 rural police workers who had retired on ill health grounds were examined.

5.3 Recruitment

5.3.1 Identification of study participants: Sheep farmers.

For practical reasons, the focus of the project was restricted to two geographical areas of England with the highest number of sheep, according to the UK Department of Environment, Food and Rural Affairs (DEFRA) ‘Distribution of Sheep in UK on 02

June 2005'. These two areas were the North and South West of England.

Three methods of sampling were used to identify the target population:

- Purposive sampling – written correspondence
- Purposive sampling – telephone contact
- Advertising

5.3.1.1 Purposive sampling – written correspondence

Contact details of farm owners in the south west and north of England were purchased from databases held by (1) a company called Experian which owns the right to sell data from the UK National Business Directory (2) a company called Tri-Direct which owns the right to sell the membership lists of the National Farmers Union (NFU) (3) The Royal Agricultural Benevolent Institution (RABI) which provides welfare advice to working and retired farmers in need, especially those who are elderly or disabled. These companies restricted the amount of contact with their members. Members could be contacted on only one occasion, by letter. It was not possible to telephone members.

Letters were sent to all of the farm owners on these databases asking them to provide us with the contact details of any sheep farmers who had retired on ill health grounds that were known to them. They were offered a small financial incentive for any contacts that subsequently met our inclusion / exclusion criteria. Eight thousand, two hundred and sixty two farm owners were contacted. The response rate was poor and less than 2% for business directories and 4.5% for RABI. Nominees identified from this method of sampling were subsequently contacted by telephone and interviewed to establish some basic facts about them including their reasons for retirement and exposure history.

5.3.1.2 Purposive sampling – telephone contact

The contact details of sheep farmers in the south west and north of England were obtained from the Wool Marketing Board (WMB). The WMB gave us this information free of charge as they were keen to assist us with this study. Over fifteen thousand farmers were listed on this database, twelve thousand of whom were new to us in the sense that they were not included on the other databases mentioned in this report. We contacted every fifth person on the WMB database, up to a total of three hundred and

ninety three farmers, by telephone and explained the purpose of this study, asked them if they had retired on ill health grounds or could suggest any other farmers who might have retired on ill health grounds. The response rate from this method of sampling was much greater than written correspondence at 59%.

5.3.1.3 Advertising

Details about this study were published in farming newspapers and publications, in organisations' newsletters such as union newsletters (NFU and Transport and General Workers Union (TGWU)) and support organisation newsletters (Organophosphate Information Network (OPIN), Pesticide Action Network (PAN) and RABI). Information about the study was also distributed at agricultural shows and sent to a number of rural GP surgeries. The study was also described in several regional radio broadcasts (circa 17) and on the Farming Today programme (twice) between 2005 and 2007.

5.3.2 Identification of study participants: Police workers.

The police force is divided into local constabularies and the Human Resources and Occupational Health Departments for each of the 12 regions we had recruited agricultural workers from were contacted and their assistance was sought in recruiting working and retired police workers into the study.

The National Association of Retired Police Officers (NARPO), which holds a database of 80,000 retired members of the police force, assisted us in recruiting rural police workers who had retired on ill health grounds. Two police convalescence and treatment centres in the UK, the Northern Police Convalescent and Treatment Centre in Harrogate and the Police Convalescent Home in Berkshire, also assisted us in recruiting rural police workers who had retired on ill health grounds.

Details of the study were emailed by police constabularies and NARPO to police workers on their database and the study was advertised in Police Press and associated websites (local police magazines and newsletters and national publications such as Police Life, Police Oracle, Police Review). Posters advertising the study were also placed in a few local police stations.

5.4 Procedures and measures

Study participants were visited at home or at their workplace and underwent an extensive neuropsychological assessment and completed a number of questionnaires to establish demographic, mood, health and exposure information.

5.4.1 Exposure history

Accurate estimation of exposure is critical for the validity of studies investigating the adverse effects of exposure to pesticides and researchers have used a range of techniques to estimate exposure in the past. Biological monitoring, such as measurement of OP metabolites in urine, of cholinesterase activity in plasma, of AChE activity in red blood cells and of neuropathy target esterase in lymphocytes, has been used to measure recent exposure but can't be used to measure cumulative long-term exposures. Epidemiological studies investigating the effects of long term exposure to pesticides often use surrogates for exposure such as ever/never worked as a farm worker or number of years spent working as a farm worker. However, these measures suffer from a number of limitations as they do not provide information about the type of pesticide a subject was exposed to, in what amount, frequency, intensity or duration, and can result in misclassification of exposure status. At the very least information in required about job title, the type of chemicals a worker was exposed to, how often and for how long.

In the present study, farm workers were asked to provide detailed information about work practices. Each sheep farmer underwent a semi-structured interview about their work and exposure history (see Appendix 2). Individuals were asked to specify when they began working with OPs, in what capacity, their level of exposure in terms of frequency and duration (i.e. number of times per year, number of days per year spent dipping sheep, flock size), 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 (i.e. 'dippers flu'), and whether or not they had felt so unwell after dipping that they sought medical help.

In addition to obtaining information about specific aspects of exposure, two exposure indices were calculated for each participant. Exposure metrics are often considered to be an improvement over conventional dichotomous exposed/non exposed categories as they attempt to consider multiple aspects of exposure history which may be relevant. A number of different exposure metrics have been proposed by researchers in the past and range from simple methods whereby different measures of frequency and duration of exposure are multiplied together to provide an overall rating; to complex formula which attempt to estimate intensity of exposure in addition to duration by incorporating weightings for variables such as job activity, use of protective clothing and so on. Exposure metrics do not reflect absolute exposure but provide a ranking within a population under study. The validity of such measures is dependent on the assumptions underlying the metric, the variables considered, the accuracy of information provided by respondents and the weighting assigned to variables.

Two metrics were calculated in this study: (1) a relatively simple estimate of 'lifetime exposure' based on the number of days per year spent using OPs multiplied by the number of years spent using OPs; (2) and a more complex formula which took account of intensity in addition to duration of exposure. The latter is referred to as the 'ESK Metric' and was devised by Cherrie and Robertson (1995) for use in a previous study of UK sheep farmers. The Esk metric takes account of the concentration of the sheep dip in the skin contamination layer (Csk) and the area of skin contaminated (Ssk), both estimated from job title, and duration of exposure (t). It involves the following formula:

$$E_{sk} = C_{sk} \text{ x } t \text{ x } S_{sk}$$

Rural police workers were only included in the study if they did not have a history of exposure to potentially toxic chemicals. This was established by telephone interview at the recruitment stage of the study. They were asked if at any time in their lives they had been exposed to or worked with potentially toxic chemicals and if they had ever felt unwell after being exposed to chemicals. Thirty three police workers had to be excluded because they had assisted farmers with sheep dipping in the past.

5.4.2 Cognitive assessment

All participants (exposed and control cohorts) underwent neuropsychological assessment. Well known, standardized and clinically sensitive tests which are

commonly used in routine clinical practice within the National Health Service were selected. All tests had adequate published reliability, validity and normative data. The Researchers who evaluated study participants were aware of which group they came from (e.g. farmer or police worker) but were blind to the participants' exposure history whilst assessing their cognitive function and mood state.

Intellectual ability - the Wechsler Adult Intelligence Scale-III was administered to assess current intellectual functioning (Wechsler, 1998). This test is composed of fourteen subtests which measure a range of cognitive functions such as general knowledge, vocabulary, arithmetic, verbal and visual reasoning, working memory, response speed and visuo-spatial ability. Two of the subtests are optional. These were not administered. In addition, the Wechsler Test of Adult Reading (WTAR) was administered to estimate premorbid intellectual ability. It estimates WAIS-III IQ index scores.

Memory - The Wechsler Memory Scale – III (short version) was used to assess working, visual and verbal memory (Wechsler, 1998). The following subtests were administered, logical memory (story recall), verbal paired associates, letter/number sequencing, digit span, spatial span, face recognition and family pictures.

Response speed and mental flexibility were assessed by a number of means (1) Trail Making Tests A and B (Spreen & Strauss, 1991) (2) The California Computer Assessment Package (CALCAP) which measures simple and choice reaction time (Miller, 2002). The Stroop test was included as a measure of mental flexibility (Trenerry, Crosson, DeBoe & Leber, 1988).

Language - the Graded Naming Test was administered to assess naming ability (McKenna & Warrington, 1983). A verbal fluency test (FAS) was used to assess expressive language (Borkowski, Benton & Spreen, 1967).

Fine Motor Skill – the Grooved Pegboard was used to assess motor dexterity (Trites, 1977). This test is very sensitive to general slowing due to medication, toxic effects and diffuse brain injury.

Effort / Malingering – The Medical Symptom Validity Test was used as a measure of cognitive effort (Green, 2004). It is a brief computerised verbal memory screening test

which measures a person's effort on testing and was included in the battery to ensure psychometric test results were valid. It is insensitive to all but the most extreme forms of cognitive impairment whilst being very sensitive to poor effort and exaggeration of cognitive difficulties.

Ten key areas of cognitive function were identified and tests were grouped in a way that would reflect participants' abilities in these areas (Table 5.2).

Table 5.2 Key cognitive domains and associated neuropsychological tests.

Cognitive Domain	*Tests*	*Cognitive Domain*	*Tests*
Working Memory	WAIS-III Digit Span	**Response Speed**	WAIS-III Digit Symbol
	WAIS-III Digit Span Backwards		Trails A
	WAIS-III Arithmetic		CALCAP Simple
	WMS-III LNS	**Verbal ability**	WAIS-III Vocabulary
			WAIS-III Comprehension
Visual Memory	WMS-III Visual Immediate memory		Graded Naming
	WMS-III Visual Delayed memory	**Verbal & visual reasoning**	WAIS-III Picture arrangement
			WAIS-III Comprehension
			WAIS-III Similarities
Auditory Memory	WMS-III Auditory Immediate memory	**Mental flexibility**	Trails B
	WMS-III Auditory Delayed memory		Stroop
			CALCAP Choice
	WMS-III Auditory Recognition delayed	**Strategy making**	Verbal Fluency
Visuo-spatial skills	WAIS-III Block design	**Fine Motor Control**	Grooved Pegboard
	WMS-III Spatial span		

5.4.3 Mood state

Mood State and Life Events – Mood state was assessed by two means: (1) A structured clinical interview (SCID; First Spitzer, Gibbon & Williams, 1997) and (2) Questionnaire measures: The Hospital Anxiety and Depression Scale (Snaith & Zigmund, 1983) and the Beck Anxiety (Beck & Steer, 1990) and Depression Inventories (Beck, Steer & Brown, 1996). The Hospital Anxiety and Depression Scale

was included to screen for clinically significant levels of anxiety and depression. Mood disorder has been shown to have a negative effect on performance on psychometric tests and so levels of anxiety and depression need to be taken account of in any analyses concerning the impact of exposure on cognitive functioning, The Beck anxiety and depression inventories were included as measures of symptom severity. However, self-assessment scales are only valid for screening purposes and definitive diagnosis requires a comprehensive clinical examination, which is why a structured clinical interview (SCID) was included in the assessment process.

Structured interviews provide an important method of standardising evaluations and improving diagnostic reliability and validity. Structured interviews standardise the coverage of specific issues (thus reducing the possibility of missed diagnoses) and require the systematic appraisal of relevant symptoms, to reduce misdiagnosis. The basic rationale for standardised interviews is the minimization of needless variability in interviewer-based evaluations. Many structured interviews have been developed over the years which vary in terms of focus and depth of coverage. The structured clinical interview (SCID; First et al, 1997) was chosen for the current study because it was developed to standardise DSM-IV evaluations of mental disorders. DSM-IV is a manual published by the American Psychiatric Association to assist clinicians in evaluating mental health disorders in both children and adults. The SCID aims to improve the reliability of DSM diagnoses and has been extensively validated over the last decade.

Study participants were evaluated for the following: Past major depressive episode, current major depressive episode, dysthymic disorder; generalised anxiety disorder, panic disorder, anxiety disorder or depression due to a general medical condition. With regard to the latter, DSM-IV requires the symptoms of anxiety or depression to be a direct physiological consequence of the general medical disorder. Anxiety and depression which has arisen as an emotional reaction to having a general medical disorder is not encompassed under this diagnostic heading.

A life events checklist (Holmes and Rahe, 1967) was included in an attempt to tease apart the relative contribution of recent stressful life events and exposure to pesticides in triggering mood disorder. This scale consists of 43 positive and negative life events such as divorce, marriage, retirement, change in financial state, which are capable of inducing stress and ill health. Each life event is assigned a value according to how

stressful they were considered to be by a standardisation sample of 394 individuals. Study participants were asked to state whether they had experienced any of these events over the last 12 months. Scores above 150 are associated with an increased risk of illness and a score above 300 is associated with a high risk of developing a stress related illness.

5.4.4 Physical health

A questionnaire concerning physical symptoms was constructed (see Appendix 2). It was based on a number of existing questionnaires with known reliability and validity: (1) the SF36 Health Survey, a multi-purpose, generic measure of disease burden (Hays, Sherbourne & Mazel, 1995) (2) the Q16 questionnaire on neurotoxic symptoms (Lundberg, Hogberg, Michelsen, Nise & Hogstedt, 1997) was examined to ensure symptoms, identified by previous research as being associated with exposure to neurotoxic agents, were included in the current questionnaire. Additional symptoms which are not associated with exposure to neurotoxic agents were added to the list such as hearing loss, toothache, tinnitus, hay fever. This was to determine whether participants who suffer from ill health show a specific pattern of disease and complain of symptoms associated with neurotoxic exposure as opposed to an array of symptoms with different underlying causes. The questionnaire was not subject to any form of further validation during this study.

Participants were asked to give an overall rating of their health and to state the degree to which their work and social life had been affected by poor health. The questionnaire then provides a list of 39 symptoms and participants were asked to state whether they suffered from these symptoms, if so, when the symptom first appeared, the frequency with which the symptom is experienced, symptom severity, level of distress caused and the degree to which the symptom interferes with daily activities.

5.4.5 Possible genetic vulnerability factors - Blood analyses (determination of PON1 status)

Genetic differences between individuals render some people more susceptible to the toxic effects of certain chemicals than others. For example, the human paraoxonase 1/arylesterase enzyme (PON1) plays an important role in the detoxification of organophosphates and helps protect against the potentially harmful effects of OPs.

Each study participant was asked to provide a sample of blood which was collected from all study participants via venipuncture following informed consent. Heparinized whole blood was centrifuged and divided into plasma, buffy coats and red blood cells and stored at -80C before being shipped in dry ice to the University of Washington, Seattle, where it was also stored at -80C until analyses of enzyme activity could take place.

PON1 status was determined under conditions that allow for determination of in vivo rates of diazoxon detoxification. An individual's PON1 status can be determined by measuring rates of paraoxon and diazoxon hydrolysis as this correlates with the amount of enzyme activity and protein levels. This method was developed and validated in adult populations by Richter & Furlong (1999 and 2009). The two-substrate enzyme analyses is carried out using molecular devices SPECTRAmax PLUS Microplate Spectrophotometer. Initial rates of substrate hydrolysis are determined with rates of diazoxon hydrolysis (y-axis) plotted against rates of paraoxon hydrolysis (x-axis). This method separates individuals into the three phenotypes, individuals homozygous for PON1 Q192, heterozygotes for PON1192 (QR) and individuals homozygous for PON1 R192. It also reveals PON1 plasma levels reported as arylesterase levels in subsequent tables and analyses.

Chapter 6 : Empirical study results

Overview

Section 1, presents the number of participants identified by different sampling methods followed by demographic information regarding the participants' who were included in the study. In Section 2 the nature and extent of cognitive impairment, mood disorder and physical health is analysed. Differences in performance between the exposed and control cohorts on psychometric testing are presented. In Section 3 the relationships between cognitive impairment and indices of exposure are described.

6.1 Section one: Recruitment rates

Initially 434 farmers came forward (222 retired, 212 working) and 252 police (170 retired, 82 working), however 67% of the farmers and 63% of the controls had to be excluded based on the inclusion/exclusion criteria or they declined to take part in the study (see Tables 6.7 and 6.8). Twelve police identified as being eligible, were not examined due to time constraints. A further 12 farmers and 3 controls were excluded to in order to establish similar demographic profiles between the groups as farmers and controls had to be matched on a number of demographic factors thought to influence cognitive performance in order to eliminate any potential confounds: age, years in education and premorbid intelligence; 5 farmers' and 1 policeman's data were excluded because they showed evidence of poor effort/malingering on a psychometric test which is insensitive to severe brain injury but which is greatly affected by effort (Green, 2004). This left a final sample of 127 exposed sheep farmers (67 working and 60 retired) and 78 unexposed controls (38 working and 40 retired).

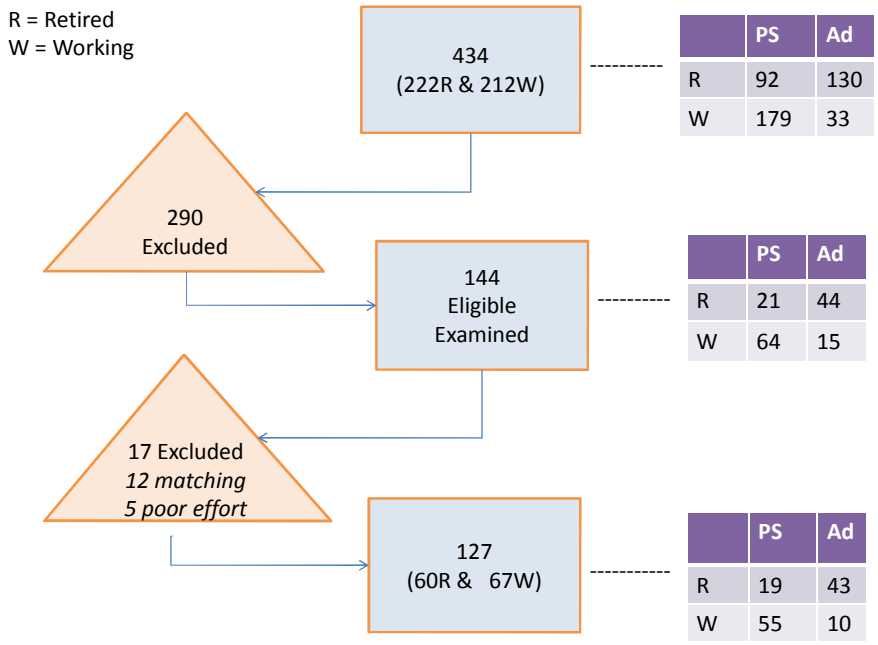


Figure 6.1 Flow diagram illustrating recruitment numbers and sources for farmers.

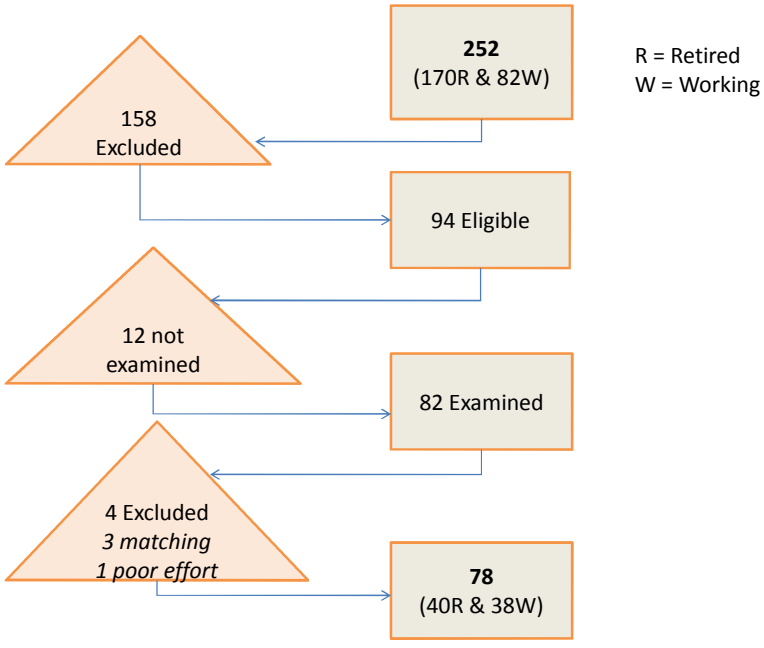


Figure 6.2 Flow diagram illustrating recruitment numbers and sources for police workers.

6.1.1 Sources of recruitment and reasons for retirement

All working and retired controls (rural police workers) were recruited by advertising. The primary reason for retirement given by police workers was musculo-skeletal injury (78%). A small number (7%) retired due to stress which had fully resolved by the time they took part in this study; and 15% retired on other ill health grounds such as breathing difficulties, gout, benign tumour, chronic fatigue and arthritis (see Table 6.6).

Working and retired farmers were recruited by a combination of purposive sampling (written correspondence and phone calls) and advertising. The majority of working farmers (81%) were recruited by purposive sampling and 19% responded to adverts. The majority of retired farmers (68%) were recruited by advert as many were no longer listed on union/business membership lists. Having said that, over a third (32%) of retired farmers were recruited by purposive sampling. Around half of the retired cohort had retired from farming completely (51%) whilst the remainder had semi- retired. None used sheep dip after retiring, the average number of years having elapsed since the last time they dipped sheep being 9 years for working farmers and 11 years for retired farmers. The primary reasons for retirement given by farmers (77%) was a constellation of non-specific symptoms including chronic fatigue, headaches, cognitive impairment, muscular and joint pain, numbness and chemical sensitivity. A quarter of these farmers attributed their symptoms to pesticide (sheep dip) poisoning. The remainder of the retired sample (23%) had retired on other ill health grounds such as musculo-skeletal injury, breathing difficulties and prostate problems (see Table 6.3).

Table 6.1 Number of farmers identified from different sampling methods.

	Written	Telephone	Support Groups	Advertising	Word of mouth	Other	Totals
Retired	25	21	27	41	10		124
Semi-retired	23	21	13	19	3	2	81
Changed occupation	1	1	2	9	3	1	17
Sub-total Retired/CO	49	43	42	69	16	3	222
Working	57	122	4	14	13	2	212

Table 6.2 Examined farmers and their source.

	Written	Telephone	Support Groups	Advertising	Word of mouth	Other	Totals
Retired	5	1	9	12	6	0	33
Semi-retired	11	4	4	10	2	1	32
Changed occupation	0	0	1	0	0	0	1
Sub-total Retired/CO	16	5	14	21	8	1	65
Working	27	37	0	6	8	1	79

Table 6.3 Primary reasons for retirement given by farmers included in the analysis.

Reason for Retirement	Included sample
Non-specific symptoms*, no attribution made	34
Non-specific symptoms attributed to OPs	16
Other ill health grounds+	15
Total	65

*Non-specific symptoms: Participants reported suffering a range of symptoms including chronic fatigue, headaches, memory loss, lack of concentration, chemical hypersensitivity, numbness, balance problems, aches and pains and symptoms of mood disorder. The majority reported a combination of several of these symptoms.

+Other ill health grounds: These included breathing difficulties, skeletal problems (back pain, arthritis), chest pain, prostate problems and poor circulation.

12 farmers were subsequently removed from the statistical analyses for age matching purposes and 5 were removed because they failed a test of effort, leaving a final sample of 127 farmers included in the analysis of cognitive function, mood state and exposure history.

Table 6.4 Number of police workers identified from different sampling methods.

	NARPO	Convalescent Homes	Advertising	Word of mouth	Totals
Retired	123	1	6	2	132
Semi-retired	4	5	1	0	10
Changed occupation	22	0	6	0	28
Sub-total Retired/CO	149	6	13	2	170
Working	7	2	65	8	82

Table 6.5 Examined police workers and their source.

	NARPO	Convalescent Homes	Advertising	Word of mouth	Totals
Retired	27	0	4	0	31
Semi-retired	1	1	0	0	2
Changed occupation	4	0	5	0	9
Sub-total Retired/CO	32	1	9	0	42
Working	6	1	29	4	40

Table 6.6 Primary reasons for retirement given by police workers included in the analysis

Reason for Retirement	Included sample
Spinal, neck, knee, shoulder, limb injury or condition	33
Reactive depression now fully resolved	3
Other ill health grounds+	6
Total	42

+Other ill health grounds include breathing difficulties, arthritis, Gout, benign tumour, Meniere's disease, chronic fatigue.

3 police workers were subsequently removed from the statistical analyses for age matching purposes and 1 was removed because he failed a test of effort, leaving a final sample of 78 police workers included in the analysis of cognitive function, mood state and exposure history.

Table 6.7 Primary reasons for exclusion - exposed cohort.

Reason for Exclusion	Retired	Semi-retired	Changed Occupation	Working	TOTAL
Acute OP poisoning	4	5		4	13
Refusal	7	5		19	31
Psychiatric	2			3	5
TBI	2	5		10	17
Neurological	15	2		1	18
CV	4	2	1		7
Epilepsy			1		1
Heart	5	6	1	2	14
Lung	2	3		3	8
Liver					
Kidney	1			1	2
IDDM	1	1		1	3
Endocrine				1	1
Cancer	4	1			5
Alcohol	5	1		3	9
Deceased	1				1
Age	21	11		16	48
Inadequate exposure history	12	9	5	35	61
Other	11	3	1	31	46
TOTAL	97	54	9	130	290

Table 6.8 Primary reason for exclusion - control cohort.

Reason for exclusion	Retired	Semi retired	Changed Occupation	Working	TOTAL
Urban	8		2	9	19
Not RIHG	17	1	1	1	20
OP exposure	25		1	7	33
Other Chemical exposure	3		1	1	5
Refusal	4		2	1	7
Age	5			10	15
Current Psychiatric	11		1		12
Retired on psychiatric	2		1		3
TBI	1			1	2
Neurological	6	1	2	1	10
CV					0
Epilepsy				1	1
Heart	2	1			3
Lung					0
Liver	1				1
Kidney	1				1
IDDM	3		2		5
Endocrine					0
Cancer					0
Alcohol	1		1		2
Area	4	1	1	7	13
Other	3		1	2	6
TOTAL:	97	4	16	41	158

6.1.2 Demographic information

Demographic characteristics of farmers and controls appear in Table 6.9. The WTAR test under estimated premorbid intelligence in both groups and was not used as a measure of premorbid ability in the analyses. Farmers and controls obtained similar scores on a test of vocabulary (which is sometimes used as a measure of premorbid ability (Lezak, 2004)), but since previous research suggests organophosphate exposed individuals may have impaired verbal ability (Mackenzie Ross et al, 2007) premorbid ability was estimated using a measure that is unlikely to have been affected by cognitive damage (matrix reasoning). Farmers and controls were successfully matched for gender, education and premorbid IQ, but not age. As the majority of psychometric test data used in the analysis were age-corrected, it is unnecessary to match the groups on age.

Table 6.9 Demographic information for the control and exposed participants.

	Exposed group Mean (SD)	*Control Group Mean (SD)*
Age*	54.73 (9.42)	51.73 (7.36)
Years in Education	11.57 (2.08)	11.95 (1.59)
Matrix Reasoning†	2.33 (.55)	2.34 (.47)
Vocabulary	10.39 (2.43)	10.99 (1.90)
Gender	102M, 25F	68M, 10F

*significant difference between groups using an independent t-test, $p<.05$
† transformed scores reported and analysed

Farmers and controls were successfully matched for gender ($\chi^2=1.61$, $p=.21$), education ($t(203)=-1.39$, $p=.17$) and premorbid IQ ($t<1$), but not age ($t(191.41)=2.54$, $p<.05$). As the majority of the tests used within the test battery were age-scaled (i.e. the effects of age were removed), the fact that the groups were not matched on age is not a problem. The potential confounding effects of age on the non-age-scaled scores will be discussed at various points in the results section.

6.2 Section 2: Exposed versus unexposed cohort comparisons; the nature and extent of cognitive impairment, mood disorder and physical ill health following exposure to OPs

This section summaries the effect of Exposure Group on neuropsychological functioning and health. To minimize any potential Type I errors Multivariate Tests of Analysis (MANOVA) were used wherever possible. When Univariate or Multivariate Analysis of Variance was used, the variables of interest were Exposure Group (which had two levels: exposed and control) and Working Status (which also had two levels: farmers and controls). When MANOVA was not appropriate[1] differences in mean scores were analysed using unrelated t -tests or the non-parametric equivalent (Mann Whitney U). Unless otherwise stated, all statistical tests were two-tailed.

Before analysis took place the distribution of all variables was checked to determine whether normality could be assumed. In order to do this, skewness (S) and kurtosis (K) values were converted into z-scores using the following equations:

$$z_{skewness} = (S-0)/SE_{skewness} \qquad z_{kurtosis} = (S-0)/SE_{kurtosis}$$

The resultant z-scores were then compared against known values for the normal distribution (see Field, 2010). In this case, as the sample size was over n=200, a value greater than 2.58 was used to signify a significantly skewed or kurtotic sample ($p<.01$)[2]. Simple tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk) were not used to determine non-normality due to the liberal nature of these tests (Field, 2010).

It was found that some variables exhibited unacceptable skewness and/or kurtosis for parametric statistical analyses. Positively skewed variables were first subjected to a square root transformation, as the action of square-rooting has a greater impact on larger numbers than smaller (which serves to bring in the right tail of the distribution towards the centre), thus reducing a positive skew. If this correction did not work, the original variables were then put through a logarithm transformation, which also brings the larger

[1] i.e. when the initial assumptions of the test were not met or when non-aged-scaled scores were being analysed

[2] Using 1.96 as the cut off criterion ($p<.05$) may be deemed too liberal in this case, as even small deviations from normality may present as significant (e.g. Field, 2010).

numbers towards the centre of the distribution. Table 6.10 shows the variables that were corrected in this way.

The same procedure was used for negatively distributed data, however in this case raw scores were reversed first by adding 1 to the highest data value in the data set and then subtracting the individual's original raw score. The addition of 1 to the highest score was to avoid producing any values of 0 (as zero has no logarithm value). To avoid confusion over the direction of the scores corrected in this way, they were then reversed back to reflect the original pattern of data (with higher numbers equating to a higher score) using the same method described above (see Table 6.10).

Table 6.10 Abnormally distributed variables.

	Square Root Transformation	Natural Logarithm Transformation	Reflected and Square Root Transformation
Digit Span	x		
Digit Span Backwards	x		
Digit-Symbol Substitution	x		
Similarites		x	
Matrix Reasoning[3]			x
Semantic fluency (animals)	x		

While it was possible to correct some of these distributions by transforming the data, transformations were not successful for all measures. The following variables were not able to be transformed into a normal distribution using the above method, and so abnormally distributed raw scores were used in the analyses:

- Trails A and B
- Grooved Pegboard (Dominant and Non-dominant hand)
- Stroop
- CALCAP (simple and choice)

[3] When the groups were matched on matrix reasoning scores, this was done using the transformed scores.

Tables 6.11-6.16 summarise the effect of Exposure Group on broad cognitive domains and the results of univariate analyses examining the effect of Exposure Group on the individual psychological tests that comprise these domains. There were no effects of Working Status (and no significant interactions), so descriptive statistics were collapsed across this variable.

6.2.1 Impaired cognitive domains

6.2.1.1 General intellectual function

While participants were matched on pre-morbid intellectual ability using matrix reasoning, an independent two-way ANOVA revealed that there was a significant main effect of Group on current IQ, as measured by the full-scale WAIS ($F(1,192)=10.21$, $p<.01$, $\eta_p^2=.05$), with the exposed group exhibiting a significantly lower IQ (mean=104.79, *11.76*) than the controls (mean=110.03, *10.83*). No effect of Working Status was found ($F<1$), nor was there a significant interaction between these variables ($F(1,192)=2.86$, $p=.09$, $\eta_p^2=.02$). Subsequent analyses reveal the exposed cohort exhibit reduced performance on specific cognitive tests (not all intellectual tests) which are associated with exposure to OPs (see following chapters) and is unlikely to reflect pre-existing differences in overall premorbid IQ between the control and exposed cohorts.

6.2.1.2 Memory

6.2.1.2.1 Working memory

Differences in working memory were analysed using Digit Span, Digit Span Backwards, Letter-Number Sequencing and Arithmetic subtests. A two way independent MANOVA revealed a significant main effect of Exposure Group on working memory ($V=.20$, $F(4,189)=12.05$, $p<.001$, $\eta_p^2=.20$), but no effect of Working Status ($V=.03$, $F(4,189)=1.54$, $p=.19$, $\eta_p^2=.03$) and no significant interaction between these variables ($F<1$). These results suggest that the exposed cohort were significantly impaired on measures of working memory and this pattern was similar for both the working and retired groups.

Follow-up univariate tests (summarized in Table 6.11) revealed that this main effect of group on working memory was driven by group differences in Digit Span, Digit Span Backwards and Letter-Number Sequencing performance.

6.2.1.2.2 Visual memory

Differences in visual memory were analysed using Visual Immediate Memory and Visual Delayed Memory. A two way independent MANOVA revealed the difference between the exposed and control participants to be a significant one (V=.07, $F(2,197)=7.54$, $p=.001$, $\eta_p^2=.07$). No effect of Working Status was found and there was no significant interaction between these variables ($F<1$ for both). To summarise, these results suggest that the exposed cohort were significantly impaired on measures of visual memory and this pattern was similar for both the working and retired groups.

Follow-up univariate tests (summarized in Table 6.11) revealed that this main effect of group on visual memory was the product of group differences in both immediate and delayed visual memory.

6.2.1.2.3 Verbal memory

Differences in auditory memory and information processing were analysed using Auditory Immediate Memory, Auditory Delayed Memory and Auditory Recognition Delayed Index Scores. A two way independent MANOVA revealed a significant multivariate effect of Exposure Group on auditory memory scores (V=.07, $F(3,197)=5.10$, $p=.002$, $\eta_p^2=.07$) which the exposed cohort performed worse than the controls. No effect of Working Status was found and there was no significant interaction between these variables (largest V=.02; largest F=1.36).

Follow-up univariate tests (summarized in Table 6.11) revealed that this main effect of group on auditory memory was the result of group differences in immediate, delayed and recognition aspects of auditory memory.

Table 6.11 IMPAIRED domains - Main effects of group on performance on memory tests and descriptive statistics and univariate effects of exposure group on cognitive performance.

	Exposed			Control				
Cognitive Area	N	Mean	SD	N	Mean	SD	Tests	FValue
Working Memory							M	12.05***
Digit Span†	121	3.04	0.38	75	3.33	0.41	A	25.12***
Digit Span Backward†	121	2.14	0.26	75	2.29	0.29	A	15.70***
Letter-Number (LNS)	121	9.55	2.62	75	11.73	2.39	A	34.53***
Arithmetic	121	11.42	2.79	75	11.48	2.89	A	0.03
Visual Memory							M	7.54***
Visual immediate	125	91.00	15.96	77	98.84	16.71	A	11.06***
Visual delayed	125	93.04	14.78	77	101.34	14.45	A	15.00***
Auditory memory							M	5.11**
Auditory immediate	125	98.86	15.89	78	107.32	13.69	A	15.35***
Auditory delayed	125	99.87	14.86	78	107.23	13.98	A	13.09***
Auditory recognition	125	101.08	15.69	78	106.03	13.30	A	5.74**

*** p<.001; ** p<.01; * p<.05; † transformed scores reported
A=ANOVA, M=MANOVA

6.2.1.3 Cognitive processing speed, control and mental flexibility

6.2.1.3.1 Response speed

Differences in response speed were analysed using Digit Symbol Substitution, CALCAP (simple) and Trails A tests. The 3 variables were analysed separately, as different statistical tests were appropriate for each one.

An independent two-way ANOVA revealed that there was a significant main effect of Group on Digit Symbol scores ($F(1,197)=32.81$, $p<.001$, $\eta_p^2=.14$), but no effect of Working Status and no significant interaction between these variables ($F<1$ for both). This suggests that the exposed cohort were significantly impaired on response speed and this pattern was similar for both the working and retired groups.

A similar deficit was found for Trails A performance, with the exposed participants performing significantly worse than the controls overall ($U=2726$, $p<.001$), and when broken down into working and retired cohorts ($U=860$, $p<.01$; $U=503$, $p<.001$ respectively).

In addition to these tests the CALCAP (simple) results were analyzed overall, and for both the working and retired cohorts. For this test participants were scored nominally by categorizing their performance as either normal or abnormal. Performance was defined as abnormal when scores lay more than 2 standard deviations away from the mean (see Table 6.12 for a breakdown of this).

Table 6.12 Breakdown of the Expected vs Observed instances of Normal and Abnormal performance on CALCAP (simple).

		Normal	Abnormal	**Total**
Total Participants				
Exposed	Observed	103	17	**120**
	Expected	107.7	12.3	
Control	Observed	63	2	**65**
	Expected	58.3	6.7	
	Total	**166**	**19**	**185**
Working Participants				
Exposed	Observed	56	6	**62**
	Expected	57.4	4.6	
Control	Observed	32	1	**33**
	Expected	30.6	2.4	
	Total	**88**	**7**	**95**
Retired Participants				
Exposed	Observed	47	11	**58**
	Expected	50.3	7.7	
Control	Observed	31	1	**32**
	Expected	27.7	4.3	
	Total	**78**	**12**	**90**

Overall, 14.2% of the exposed participants performed abnormally on this test, compared to 3.1% of the controls, which was found to be significant ($\chi^2(1)= 5.63$, $p<.05$). However, when broken down for the two working status groups, this difference was only found to be significant for the retired cohort (working: $\chi^2(1)=1.39$, *ns*; retired: $\chi^2(1)= 4.48$, $p<.05$).

To summarise, the exposed cohort appear to be impaired on measures of response speed.

6.2.1.3.2 Fine motor control

As organophosphates can damage the peripheral nerves, a measure of fine motor control was included into the test battery in the form of the grooved peg board. Mann Whitney U tests were used to investigate whether there was a significant deficit in the exposed cohort.

Overall, the exposed participants were found to perform significantly worse than the controls on the Grooved Peg Board for both the dominant ($U=2235$, $p<.001$) and non-

dominant hand (U=2554.50, p<.001).

When broken down into working status the same pattern was found for the working (dominant hand: U=468, p<.001; non-dominant hand: U=610.50, p<.001) and retired cohort (dominant hand: U=562.50, p<.001; non-dominant hand: U=606., p=.001).

6.2.1.3.3 Executive function

Participants' performance on measures of executive function were examined in three ways: 1.) tests of mental flexibility and participant's ability to switch between tasks by successfully inhibiting responses to certain stimuli were measured using CALCAP-choice, Trails B and Stroop. 2.) Participants' ability to use successful strategies to complete tasks was examined using Verbal Fluency. 3.) Participants' Verbal and Visual Reasoning Skills were examined using Picture Arrangement, Comprehension and Similarities.

6.2.1.3.3.1 Mental flexibility & inhibition

Mann Whitney U tests revealed that the exposed participants were significantly impaired on Trails B compared to matched controls overall (U=2835.5, p<.001), and when broken down into working and retired cohorts (U=769.5, p=.001; U=643.5, p<.001 respectively).

On CALCAP (choice) 24.2% of the exposed participants exhibited abnormal performance, compared to 9.2% of the controls, which was a significant difference ($\chi^2(1)=6.13$, p<.05). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=.90$, *ns*; retired: $\chi^2(1)= 6.11$, p<.05).

Table 6.13 Breakdown of the Expected vs Observed instances of Normal and Abnormal performance on CALCAP (choice).

		Normal	Abnormal	**Total**
Total Participants				
Exposed	Observed	91	29	**120**
	Expected	97.3	22.7	
Control	Observed	59	6	**65**
	Expected	52.7	12.3	
	Total	**150**	**35**	**185**
Working Participants				
Exposed	Observed	52	10	**62**
	Expected	53.5	8.5	
Control	Observed	30	3	**33**
	Expected	28.5	4.5	
	Total	**82**	**13**	**95**
Retired Participants				
Exposed	Observed	39	19	**58**
	Expected	43.8	14.2	
Control	Observed	29	3	**32**
	Expected	24.2	7.8	
	Total	**68**	**22**	**90**

In addition to this the Stroop test was examined. The Stroop test is a measure of mental flexibility, in particular, the ability to switch between competing response modes. The manual that accompanies the Stroop neuropsychological screening test provides normative data for healthy and brain damaged populations and cutoff scores for abnormality, stratified by age.

The analysis revealed that 14.2% of the exposed participants failed the Stroop test, compared to only 1.5% of the controls, which was a significant difference ($\chi^2(1)=15.84$, $p<.001$). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=5.65$, $p<.06$; retired: $\chi^2(1)= 11.77$, $p<.01$).

Table 6.14 Breakdown of the Expected vs Observed instances of passes and fails on the Stroop.

		Pass	Fail	**Total**
Total Participants				
Exposed	Observed	94	29	**123**
	Expected	104.7	19.8	
Control	Observed	75	3	**78**
	Expected	64.3	12.2	
	Total	**169**	**32**	**201**
Working Participants				
Exposed	Observed	49	16	**65**
	Expected	53.6	12.1	
Control	Observed	35	3	**38**
	Expected	30.4	6.9	
	Total	**84**	**19**	**103**
Retired Participants				
Exposed	Observed	45	13	**58**
	Expected	51	7.8	
Control	Observed	40	0	**40**
	Expected	34	5.2	
	Total	**85**	**13**	**98**

To summarise, the above results suggest that the exposed cohort were significantly impaired on measures of mental flexibility and that this pattern was more pronounced in the retired group.

6.2.1.3.3.2 Strategy making

An independent two-way ANOVA revealed that there was a significant main effect of Group on verbal fluency scores ($F(1,200)=50.21$, $p<.001$, $\eta_p^2=.20$), but no effect of Working Status ($F<1$) and no significant interaction between these variables ($F(1,200)=3.61$, $p=.06$, $\eta_p^2=.02$). This suggests a deficit in strategy making ability in the exposed cohort, with a similar pattern for both the working and retired groups. However, it is of note that Levene's test of homogeneity of variance was significant in this case ($F(3,200)=4.02$, $p<.01$), thus these findings should be interpreted with caution.

Mann Whitney U tests (which do not assume equality of variance) confirmed this pattern, with the exposed group performing significantly worse that the controls on verbal fluency overall ($U=2244$, $p<.001$), and when broken down into the working and retired cohorts ($U=655.5$, $p<.001$; $U=479$, $p<.001$ respectively).

Table 6.15 IMPAIRED domains - Main effects of group on performance on tests of response speed and mental flexibility and descriptive statistics and univariate effects of exposure group on cognitive performance.

	Exposed			Control				
Cognitive Area	N	Mean	SD	N	Mean	SD	Tests	FValue
Response speed								
Digit Symbol	126	2.94	0.42	75	3.27	0.33	A	32.81***
Trails A	127	41.03	15.30	74	32.27	13.05	U	2726***
CALCAP simple							X^2	see text
Fine Motor Control								
Grooved Pegboard Dominant Hand (RT)	124	92.65	24.51	74	75.08	11.89	U	2235***
Grooved Pegboard Non-Dominant Hand (RT)	123	96.49	22.47	73	81.12	13.92	U	2554.5***
Mental Flexibility								
Trails B	124	96.26	44.74	77	71.03	30.79	U	2835.5***
Stroop							X^2	see text
CALCAP choice							X^2	see text
Strategy Making								
Verbal fluency	127	32.88	11.51	77	44.21	10.47	A	50.21***

*** p<.001; ** p<.01; * p<.05

A=ANOVA, U=Mann-Whitney, X^2=Chi Square

6.2.2 Intact cognitive domains

6.2.2.1 Verbal and visuo-spatial ability and reasoning skills

6.2.2.1.1 Verbal ability

Participants' performance on verbal ability was assessed using Vocab, Graded Naming and Comprehension. A two way independent MANOVA revealed no significant main effects or interactions (largest V=.03, largest F=1.81). These results suggest that verbal abilities are largely intact.

6.2.2.1.2 Visuo-spatial abilities

Differences in visuo-spatial abilities were analysed using Block Design and Spatial Span. A two way independent MANOVA revealed no significant main effects or interactions (largest V=.02, largest F=1.65). These results suggest that visuo-spatial abilities are largely intact.

6.2.2.1.3 Verbal & visual reasoning

In order to investigate whether there was a significant effect of Exposure Group or Working Status on Verbal and Visual Reasoning Skills, scores on Similarities, Comprehension and Picture Arrangement were entered into a two way independent multivariate analysis of variance (MANOVA). Pillai's trace revealed no significant main effects or interactions (highest V =.04; highest F=2.27).

Table 6.16 INTACT domains - Main effects of group on performance on tests of verbal and visuo-spatial ability, reasoning and general intellectual function and descriptive statistics and univariate effects of exposure group on cognitive performance.

	Exposed			Control				
Cognitive Area	N	Mean	SD	N	Mean	SD	Tests	FValue
Verbal Ability							M	1.81
Vocabulary	119	10.39	2.43	73	10.99	1.90	A	3.45
Comprehension	119	10.84	2.64	73	11.41	2.05	A	2.75
Graded Naming	119	12.41	1.28	73	12.78	1.12	A	4.41*
Visuo-spatial ability							M	1.65
Block Design	125	11.98	2.84	76	12.17	3.19	A	0.22
Spatial Span	125	10.10	2.64	76	10.83	3.14	A	3.21
Verbal/Visual Reasoning							M	2.45
Picture Arrangement	119	9.92	2.70	69	10.96	2.78	A	6.58*
Similarities	119	2.32	0.25	69	2.37	0.17	A	2.75
Comprehension	119	10.84	2.64	69	11.28	2.13	A	1.66

*** p<.001; ** p<.01; * p<.05

A=ANOVA, M=MANOVA

6.2.3 Summary

Overall, the results seen in this chapter illustrate a pattern of patchy under functioning in some, but not all areas of cognitive performance in the exposed cohort. These findings are summarised in Table 6.17.

Table 6.17 Areas of cognitive deficit in the exposed cohort

Cognitive Domain	**Function Status**
General Intellectual Ability	*Impaired*
Working Memory	*Impaired*
Visual Memory	*Impaired*
Auditory Memory	*Impaired*
Response Speed	*Impaired*
Fine Motor Control	*Impaired*
Mental Flexibility & Inhibition	*Impaired*
Strategy Making	*Impaired*
Verbal Ability	*Intact*
Visio-Spatial Abilities	*Intact*
Verbal & Visual Reasoning Ability	*Intact*

While the results suggest a that long-term, low-level exposure to OPs may cause a significant pattern of cognitive deficit, it does not take into account possibility that the observed finding may have been due to other, extraneous variables inherent in the exposed cohort (but not the control group). Indeed, it may be that the pattern of results could be explained by other variables intrinsic to the exposed cohort that have not been considered in this analysis. As such, the next chapter will address a specific set of possible covariates known to influence cognitive function, in order to assess the validity of the above findings. Specifically, the next chapter will seek to address the following questions:

(1) Could the pattern of deficit be due to mood disorder rather than exposure to OPs?

(2) Could the pattern of deficit observed above have been driven by inclusion of study participants with undiagnosed acute exposure?

Chapter 7 Further analyses: Exploring the exposed cohort

7.1 Could the pattern of deficit observed be due to mood disorder rather than exposure to OPs? Re-analysis of the data controlling for the effects of mood.

Previous research has shown that depression and anxiety can significantly alter cognitive performance (Baddeley, Wilson & Watts, 1995). 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.

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 some information-processing speed and memory tests (prose recall, list learning, design learning, but working memory is usually intact; Watts, 1996; Zakzanisi, Leach and Kaplan, 1998), mood disorder is associated with a lesser degree of impairment than organic brain damage (Coughlan and Hollows, 1984; Watts, 1996). 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, 1995). Impaired functioning is more common in patients with moderate to severe depression, in older patients (mean age more than 50 years) and those who take antidepressant medication (Austin, et al, 1999; Paradisio, Lamberty, Garvey & Robinson, 1997; Zakzanisi 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 & Young, 2003).

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).

This chapter intends to address the question of whether the observed cognitive deficit seen in the farming cohort in the previous chapter is due to mood disorder rather than exposure to OPs. Study participants were asked to complete the Hospital Anxiety and Depression Scale (HAD), a screening tool for anxiety and depression which was designed for use with patients who may suffer concurrent physical illness. Figure 7.1 shows the proportion of participants in each group with clinically significant depression and anxiety scores on the HAD. 45.9% of farmers had scores above clinical cut-offs for depression compared to only 6.6% controls; and 41 % of farmers scored in the clinical range for anxiety compared to only 22.4% of controls. The incidence of depression and anxiety was particularly high amongst retired farmers.

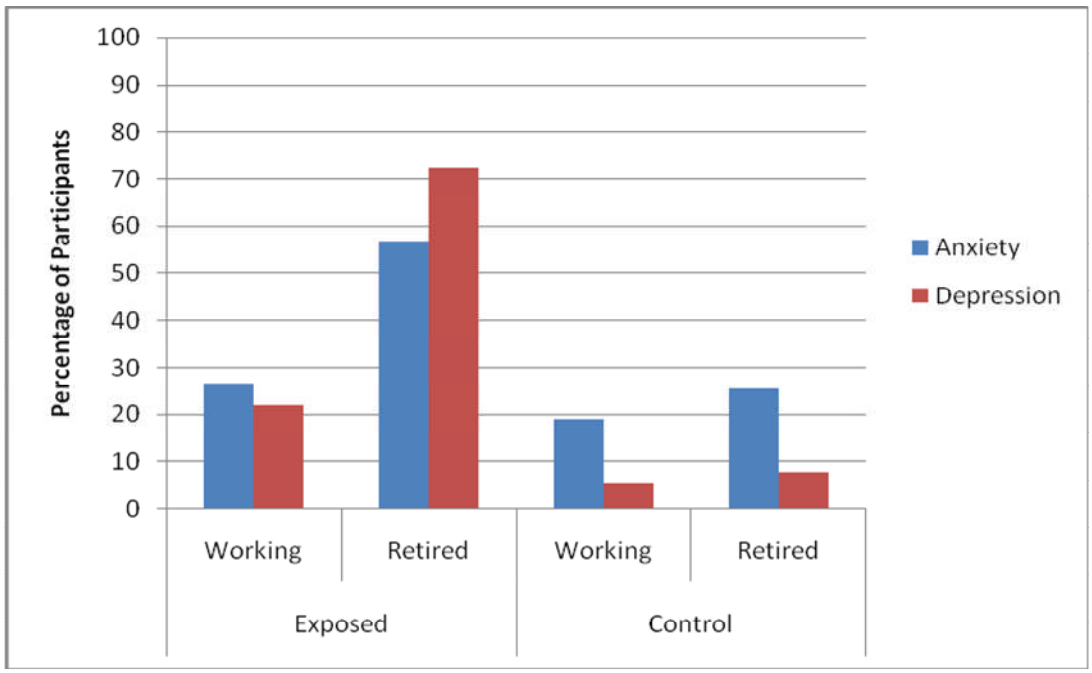


Figure 7.1 Proportion of participants in each group with clinically significant depression and anxiety scores on the Hospital Anxiety and Depression Scale.

Table 7.1 Mean scores on the Hospital Anxiety and Depression Scale for the controls and exposed participants in both the working and retired groups.

			HADS Anxiety Score			HADS Depression Score		
		N	Range	Mean	SD	Range	Mean	SD
Exposed	Working	64	0-16	6.19	4.15	0-15	4.61	3.76
	Retired	58	3-17	8.98	4.09	0-17	9.40	4.15
Control	Working	37	0-10	3.76	3.09	0-8	2.41	2.34
	Retired	39	0-14	5.03	3.41	0-9	3.28	2.74

Table 7.1 shows the mean HAD scores for the different groups. As all anxiety and depression scores were distributed abnormally, as series of Mann Whitney U tests were carried out to investigate group differences for the working and retired cohorts. Exposed participants obtained significantly higher scores on the depression subscale of the HAD than the controls for both the working and retired cohorts ($U=762.5$, $p<.01$; $U=267$, $p<.001$ respectively) and the same was true for anxiety scores (HADS working: $U=759.5$, $p<.01$; HADS retired: $U=536.5$, $p<.001$).

As previous research has shown that depression and anxiety may be related to poor performance on certain psychometric tests (Baddeley, Wilson & Watts, 1995) and as there are differences in mood scores between the different exposure and employment groups, it is therefore standard practice to take account of participants' mood scores when analysing their performance on neuropsychological tests.

In order to do this the original analyses on cognitive performance were re-run with the effects of mood partialled out. This was done by re-running the above ANOVAs and MANOVAs with age scaled variables, but by including depression and anxiety scores as covariates. While all depression and anxiety scores were extremely skewed and kurtotic, the HADS anxiety and depression scores were able to be normalised using a simple squareroot transformation (a constant of 1 was added to all raw scores as the lowest score was 0, prior to transformation). As such, these transformed variables were used to control for mood and anxiety in the parametric tests.

Re-analysis of the non-parametric tests are described separately as a different method to control for depression and anxiety was used. In this case, participants with any signs of depression and/or anxiety according to the HAD scale were removed from the analysis entirely.

7.1.1 Originally impaired cognitive domains

7.1.1.1 Intellectual function

An independent two-way ANCOVA (with depression and anxiety scores on the HADS as covariates) revealed that there was a significant main effect of Group on current IQ, as measured by the full-scale WAIS ($F(1,184)=7.57$, $p<.01$, $\eta_p^2=.04$), with the exposed group exhibiting a significantly lower IQ than the controls even after controlling for mood and anxiety levels. No relationship was found between the covariates and IQ ($F<1$ for both) and no effect of Working Status was found ($F<1$). There was also no significant interaction between these variables ($F(1,192)=2.33$, $p=.13$, $\eta_p^2=.01$). Subsequent analyses reveal the exposed cohort exhibit reduced performance on specific cognitive tests (not all intellectual tests) which is associated with exposure to OPs (see following chapters) and is unlikely to reflect pre-existing differences in overall premorbid IQ between the control and exposed cohorts.

7.1.1.2 Working memory

Differences in working memory were re-analysed using Digit Span, Digit Span Backwards, Letter-Number Sequencing and Arithmetic subtests and with HADS depression and anxiety scores as covariates. A two way independent MANCOVA, revealed that neither HADSA ($V=.02$, $F<1$) nor HADSD scores ($V=.03$, $F(4,181)=1.45$, $p=.22$, $\eta_p^2=.03$) were significantly related to working memory; and after controlling for the effect of these variables, a significant main effect of Exposure Group on working memory was found ($V=.16$, $F(4,181)=8.48$, $p<.001$, $\eta_p^2=.16$). Again, no effect of Working Status ($V=.02$, $F(4,181)=1.08$, $p=.37$, $\eta_p^2=.02$) and no significant interaction between the independent variables ($V=.02$, $F(4,181)=1.08$, $p=.37$, $\eta_p^2=.02$) were found. These results suggest that the exposed cohort were significantly impaired on measures of working memory, even after the effects of mood and anxiety had been partialled out; and this pattern was similar for both the working and retired groups.

Follow-up univariate tests, also controlling for HADSA and HADSD scores (summarized in Table 7.2) revealed that this main effect of group on working memory was driven by group differences in Digit Span, Digit Span Backwards and Letter-Number Sequencing performance.

7.1.1.3 Visual memory

As with working memory, the effect of exposure and working status on visual memory was re-analysed with HADS depression and anxiety scores as covariates. Using Visual Immediate Memory and Visual Delayed Memory as dependent variables, a two way independent MANCOVA revealed no significant relationship between either depression or anxiety and visual memory (largest V=.002, F<1 for both). Again, the difference between the exposed and control participants was found to be significant (V=.07, F(2,189)=6.97, p=.001, η_p^2=.07), even after removing the potential effect of mood and anxiety. No effect of Working Status was found (V=.01, F(2,189)=1.07, p=.34, η_p^2=.01) and there was no significant interaction between these variables (F<1). To summarise, these results suggest that after controlling for mood and anxiety, the exposed cohort were significantly impaired on measures of visual memory and this pattern was similar for both the working and retired groups.

Follow-up univariate tests (summarized in Table 7.2) revealed that this main effect of group on visual memory was the product of group differences in both immediate and delayed visual memory (again, after partialling out HADS anxiety and depression scores).

7.1.1.4 Auditory verbal memory

Differences in auditory memory and information processing were re-analysed using Auditory Immediate Memory, Auditory Delayed Memory and Auditory Recognition Delayed Index Scores as dependent variables, and HADSA and HADSD scores as covariates. A two way independent MANCOVA revealed no significant relationship between auditory memory and depression (F<1) or anxiety (V=.02, F(3,189)=1.28, p=.28, η_p^2=.02). After controlling for these effects, a significant multivariate effect of Exposure Group on auditory memory was found (V=.06, F(3,189)=4.05, p<.01, η_p^2=.06), in which the exposed cohort performed worse than the controls. No effect of Working Status was found and there was no significant interaction between these variables (largest V=.02; largest F=1.12).

Follow-up univariate tests controlling for the effect of depression and anxiety (summarized in Table 7.2) revealed that this main effect of group on auditory memory was the result of group differences in immediate, delayed and recognition aspects of

auditory memory.

7.1.1.5 Response speed

Differences in response speed were analysed parametrically using Digit Symbol Substitution. An independent two-way ANCOVA using HADSA and HADSD scores as covariates revealed that there was no significant relationship between Digit Symbol scores and either anxiety ($F(1,188)=1.44$, $p=.23$, $\eta_p^2<.01$) or depression ($F(1,188)=2.92$, $p>.05$, $\eta_p^2=.01$) scores. The significant main effect of Group on Digit Symbol scores held true ($F(1,188)=19.87$, $p<.001$, $\eta_p^2=.10$) even after the influence of mood was partialled out. No effect of Working Status and no significant interaction between these variables was found ($F<1$ for both). This suggests that even after controlling for the possible influence of depression and anxiety, the exposed cohort were significantly impaired on response speed and this pattern was similar for both the working and retired groups.

7.1.1.5.1 Response speed: Non-parametric analyses

As response speed measures Trails A and CALCAP (simple) cannot be analysed parametrically (see Section 6.2) mood and anxiety needed to be controlled for in a different manner for these variables. To try and remove the possible influence of mood on cognitive ability, the participants were split into groups according to their scores on the Hospital Anxiety and Depression Scales. The HAD is a diagnostic screening tool and the standard cutoff score of 8 was used to determine clinical caseness. Score below 8 are considered normal and participants who obtained scores greater than 8 on either the depression or anxiety subscale were removed from the analyses. Non-parametric analyses were then rerun, having removed the influence of mood, with the following outcomes:

Mann-Whitney U tests on Trails A performance found that the exposed participants performed significantly worse than the controls for the working group ($U=423.5$, $p<.05$), however this did not reach significance in the retired cohort ($U=79$, $p=.06$).

With regard to simple reaction time, 9.6% of exposed participants performed abnormally on the CALCAP (simple) test, compared to 0% of controls, which was found to be significant ($\chi^2(1)= 5.06$, $p<.05$). However, when broken down for the two working status groups, this difference was only found to be significant for the retired

cohort (working: $\chi^2(1)=2.02$, *ns*; retired: ($\chi^2(1)= 4.39$, $p<.05$).

The above results indicate that response speed deficits were evident in the exposed cohort, even after controlling for the potentially confounding effects of depression and anxiety.

7.1.1.6 Fine motor control

As Grooved Peg Board scores could not be analysed parametrically, analysis controlled for mood and anxiety using the same method outlined in the previous section.

Mann-Whitney U tests on Grooved Peg Board scores for the dominant hand revealed that the exposed participants were found to perform significantly worse than the controls in both the working and retired cohorts (U=246, p<.001; U=67, p<.05 respectively). This pattern was replicated for non-dominant hand scores (working: U=315.5, p=.001; retired: U=71, p<.05). Overall, this suggests an impairment in fine motor control for the exposed cohort after controlling for mood and anxiety issues.

7.1.1.7 Executive function: Strategy making

An independent two-way ANCOVA with anxiety and depression scores as covariates revealed that there was no relationship between verbal fluency and HAD depression scores (F<1), but there was a significant relationship with anxiety (F(1,191)=4.68, p<.05, η_p^2=.02). Nevertheless, a significant main effect of Group on verbal fluency scores was still found (F(1,191)=46.93, p<.001, η_p^2=.20) even after the effects of mood were partialled out. Again, no effect of Working Status (F<1) was found. However in this case there was a significant interaction between these variables was found (F(1,191)=4.01, p>.05, η_p^2=.02), with working farmers performing better than retired farmers (means=33.52 and 30.84 respectively), while the opposite pattern was observed in the control group (working control mean = 42.97; retired control mean = 46.97). This suggests a deficit in strategy making ability in the exposed cohort even after controlling for anxiety and depression, however the reason behind the interaction with working status is unclear.

7.1.1.8 Executive function: Mental flexibility & inhibition

Non-parametric analyses were carried out controlling for mood in the way outlined in section 7.1.2.5.1. Mann-Whitney U tests on Trails B performance found that the exposed participants performed significantly worse than the controls for both the working and retired cohorts (U=385.5, p<.01; U=67, p<.05 respectively).

With regard to choice reaction time, 15.4% of exposed participants performed abnormally on CALCAP (choice), compared to 12% of controls and no significant difference was found for between exposed and control subjects or working status groups ($\chi^2(1)= 0.25$, *ns*; working: $\chi^2(1)<1$, *ns*; retired: $\chi^2(1)= 1.35$, *ns*).

With regard to mental flexibility, 22.2% of the exposed participants failed the Stroop test, compared to only 1.8% of the controls, which was a significant difference ($\chi^2(1)=11.24$, p<.001), for the two working status groups (working: $\chi^2(1)=5.08$, p<.05; retired: $\chi^2(1)= 5.91$, p<.05).

While differences in CALCAP (choice) scores did not differ according to exposure, significant deficits were evident for mental flexibility and inhibition in terms of Trails B and Stroop, indicating that the exposed cohort are impaired on this dimension, even after controlling for depression and anxiety.

Table 7.2 Impaired domains after including depression and anxiety scores and covariates in parametric analyses.

	Exposed			Control				
Cognitive Area	N	Mean	SE	N	Mean	SE	Tests	FValue
Working Memory							Mc	8.48***
Digit Span†	117	3.04	0.04	73	3.34	0.05	Ac	20.15***
Digit Span Backward†	117	2.14	0.03	73	2.28	0.03	Ac	8.58**
Letter-Number (LNS)	117	9.54	0.25	73	11.70	0.32	Ac	24.83***
Arithmetic	117	11.31	0.27	73	11.74	0.35	Ac	0.80
Visual Memory							Mc	6.97***
Visual immediate	121	90.79	1.59	75	99.79	2.06	Ac	10.55***
Visual delayed	121	93.15	1.42	75	102.34	1.84	Ac	13.81***
Auditory memory							Mc	4.05**
Auditory immediate	121	99.05	1.44	76	107.01	1.86	Ac	10.05***
Auditory delayed	121	99.44	1.37	76	107.75	1.77	Ac	12.08***
Auditory recognition	121	100.99	1.45	76	105.88	1.87	Ac	3.75*
Response speed								
Digit Symbol †	121	2.96	0.04	73	3.25	0.05	Ac	19.87***
Strategy Making								
Verbal fluency	122	32.18	1.06	75	44.97	1.39	Ac	46.93***

*** p<.001; ** p<.01; * p=.05; † transformed scores reported
Ac=ANCOVA, Mc=MANCOVA

7.1.2 Summary

To examine whether the patterns of deficit observed in the initial analysis could have been driven by the potentially confounding effects of anxiety and depression on cognitive performance, the data was re-analysed after controlling the effects of mood statistically or removing all farmers who scored in the clinical range for anxiety and/or depression on the HAD scale. The same areas of deficit remained after the effects of anxiety and depression were removed, with only three minor changes, involving the influence of working status, but not exposure group, on tests of executive function and motor speed (stroop, verbal fluency and Trails A); and one significant exception which involved choice reaction time results. In the initial analyses a significant difference was found between exposed and control subjects on CALCAP choice results, but this difference was lost during follow-up analyses which controlled for mood.

7.2 Could the pattern of deficit observed above have been driven by inclusion of study participants with undiagnosed acute exposure? Re-analysis of the data after removal of participants who report a history of 'dippers flu'.

The previous section sought to address the question of whether cognitive deficits evident in the farming cohort were secondary to mood disorder. The findings illustrated that, generally, the original deficit pattern seen in Chapter 6 remained, even after controlling for mood. While this suggests the cognitive deficits may be a product of long-term low level exposure to organophosphates, another fundamental question needs to be addressed before this explanation can be accepted: whether the observed deficits are due to accidental inclusion of study participants with a history of acute exposure.

The aim of the current study is to determine whether repeated low level exposure to OPs is harmful to human health, so individuals with a history of high level exposure and acute poisoning were excluded. However, the definition of acute poisoning is that which results in symptoms of acute toxicity of such severity that people seek medical help. However, research has shown that variables other then severity of illness determine whether individuals consult physicians (Pitts & Phillips, 1991) and the incidence of GP visits per capita are notoriously low in the country (British Medical Association, 2005). It may be a mistake to rely on measures of acute exposure which are defined by medical help-seeking behaviour as a definition of this kind may under-estimate instances of acute poisoning. Instead, it may be prudent to consider symptom-based diagnostic criteria for acute exposure. A number of participants (33.8% of the working cohort and 50.9% of the retired group) reported that throughout their working life they suffered repeated episodes of flu-like symptoms 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. Hence, 'dippers flu' may reflect undiagnosed, untreated acute toxicity (Cherry, Mackness, Mackness, Dippnall & Povey, 2011).

The aim of this section is to determine whether the observed pattern of cognitive deficits seen in Chapter 6 could be explained by inclusion of participants with

undiagnosed acute exposure. To examine whether the patterns of deficit observed in the initial analysis could have been driven by participants with undiagnosed acute exposure, the data was re-analysed after removing all farmers who had experienced 'dippers flu' (and those for which this information was not available, n=5). This left a total of 43 working and 28 retired farmers. A chi-square test revealed no significant difference between working and retired farmers in terms of how many reported 'dippers flu' (χ^2 (1)= 3.62, *ns*).

The results are described below.

7.2.1 Originally impaired cognitive domains

7.2.1.1 General intellectual function

While participants were matched on pre-morbid intellectual ability using matrix reasoning, an independent two-way ANOVA revealed that there was a significant main effect of Group on current IQ, as measured by the full-scale WAIS ($F(1,139)=8.66$, $p<.01$, $\eta_p^2=.06$), with the exposed group exhibiting a significantly lower IQ than the controls. No effect of Working Status was found ($F<1$), however the interaction between these variables reached marginal significance ($F(1,139)=3.92$, $p=.05$, $\eta_p^2=.03$). This suggests that the exposed cohort were significantly impaired on general intellectual functioning. While the working cohort had a higher IQ score than the retired participants in the exposed group (means = 106.69; 101.74 respectively), the opposite was true for the controls (working mean = 108.61; retired mean = 111.53).

7.2.1.2 Working memory

Differences in working memory were analysed using Digit Span, Digit Span Backwards, Letter-Number Sequencing and Arithmetic subtests. A two way independent MANOVA revealed a significant main effect of Exposure Group on working memory ($V=.19$, $F(4,136)=8.07$, $p<.001$, $\eta_p^2=.19$), but no effect of Working Status ($V=.03$, $F(4,136)=1.06$, $p=.38$, $\eta_p^2=.03$) and no significant interaction between these variables ($F<1$). These results suggest that the exposed cohort were significantly impaired on measures of working memory and this pattern was similar for both the working and retired groups.

Follow-up univariate tests (summarized in Table 7.3) revealed that this main effect of group on working memory was driven by group differences in Digit Span, Digit Span Backwards and Letter-Number Sequencing performance.

7.2.1.3 Visual memory

Differences in visual memory were analysed using Visual Immediate Memory and Visual Delayed Memory. A two way independent MANOVA revealed the difference between the exposed and control participants to be a significant one (V=.08, $F(2,142)=6.41$, $p=.002$, $\eta_p^2=.08$). No effect of Working Status was found and there was no significant interaction between these variables ($F<1$ for both). To summarise, these results suggest that the exposed cohort were significantly impaired on measures of visual memory and this pattern was similar for both the working and retired groups.

Follow-up univariate tests (summarized in Table 7.3) revealed that this main effect of group on visual memory was the product of group differences in both immediate and delayed visual memory.

7.2.1.4 Auditory memory and information processing

Differences in auditory memory and information processing were analysed using Auditory Immediate Memory, Auditory Delayed Memory and Auditory Recognition Delayed Index Scores. A two way independent MANOVA revealed a significant multivariate effect of Exposure Group on auditory memory and information processing scores (V=.07, $F(3,142)=3.56$, $p<.05$, $\eta_p^2=.07$) which the exposed cohort performed worse than the controls. No effect of Working Status was found and there was no significant interaction between these variables (largest V=.04; largest F=2.14).

Follow-up univariate tests (summarized in Table 7.3) revealed that this main effect of group on auditory memory was the result of group differences in immediate, delayed and recognition aspects of auditory memory.

7.2.1.5 Response speed

Differences in response speed were analysed using Digit Symbol Substitution, CALCAP (simple) and Trails A tests. The 3 variables were analysed separately, as different statistical tests were appropriate for each one.

An independent two-way ANOVA revealed that there was a significant main effect of Group on Digit Symbol scores ($F(1,142)=29.82$, $p<.001$, $\eta_p^2=.17$), but no effect of Working Status and no significant interaction between these variables (largest $F=1.10$). This suggests that the exposed cohort were significantly impaired on response speed and this pattern was similar for both the working and retired groups.

A similar deficit was found for Trails A performance, with the exposed participants performing significantly worse than the controls in both the working and retired cohorts ($U=583.5$, $p<.05$; $U=208$, $p<.001$ respectively).

With regard to simple reaction time, 6.8% of exposed participants performed abnormally on the CALCAP (simple) test, compared to 1.5% of controls, which was found to be significant ($\chi^2(1)= 4.63$, $p<.05$). However, when broken down for the two working status groups, this difference was only found to be significant for the retired cohort (working: $\chi^2(1)=1.38$, *ns*; retired: $\chi^2(1)= 3.80$, $p=.05$).

To summarise, the exposed cohort appear to be impaired on measures of response speed.

7.2.1.6. Fine motor control

As before, scores on the Grooved Peg Board task were analysed for both the dominant and non-dominant hand).

When broken down into working status the exposed participants were found to perform significantly worse than the controls for both hands, in both the working (dominant hand: $U=345$, $p<.001$; non-dominant hand: $U=383.5$, $p<.001$) and retired cohorts (dominant hand: $U=228.5$, $p<.001$; non-dominant hand: $U=218$, $p<.001$).

7.2.1.7 Executive function: Mental flexibility & inhibition

Mann Whitney U tests revealed that the exposed participants were significantly impaired on Trails B in both the working and retired cohorts ($U=515$, $p<.01$; $U=294$, $p<.01$ respectively).

With regard to choice reaction time, 11.4% of exposed participants performed abnormally on CALCAP (choice), compared to 4.5% of controls which was found to be significant ($\chi^2(1)=4.27$, $p<.05$). However, when broken down for the two working status

groups, this difference was only found to be significant for the retired cohort (working: $\chi^2<1$ *ns*; retired: $\chi^2(1)= 7.96$, $p=.01$).

With regard to mental flexibility, 11.4% of the exposed participants failed the Stroop test, compared to only 2% of the controls, which was a significant difference ($\chi^2(1)=14.28$, which occurred in both of the working status groups (working: $\chi^2(1)=6.50$, $p<.05$; retired: $\chi^2(1)=7.71$, $p<.01$).

To summarise, the above results suggest that the exposed cohort were significantly impaired on measures of mental flexibility and that this pattern was more pronounced in the retired group (based on CALCAP analyses).

7.2.1.8 Executive function: Strategy making

An independent two-way ANOVA revealed that there was a significant main effect of Group on verbal fluency scores ($F(1,144)=30.23$, $p<.001$, $\eta_p^2=.17$), but no effect of Working Status ($F(1,144)=2.38$, $p=.13$, $\eta_p^2=.02$) and no significant interaction between these variables ($F<1$). This suggests a deficit in strategy making ability in the exposed cohort, with a similar pattern for both the working and retired groups. However, it is of note that Levene's test of homogeneity of variance was significant in this case ($F(3,144)=4.48$, $p<.01$), thus these findings should be interpreted with caution.

7.2.2 Summary

To examine whether the patterns of deficit observed in the initial analysis could have been driven by unintentional inclusion of participants with a history of undiagnosed acute exposure, the data was re-analysed after removing all farmers who had experienced 'dippers flu'. The same areas of deficit remained even after removal of these subjects with only two minor changes, one involving the influence of working status, but not exposure group, on a test of executive function (Stroop); and one involving an interaction between working status and exposure group in relation to overall, full scale IQ. These findings indicate that the pattern of deficit observed in the initial analyses was not driven by inclusion of participants with undiagnosed acute toxicity.

Table 7.3 Main effects of group on parametric tests of cognitive performance that were originally impaired, once possible acute exposure had been removed.

	Exposed			Control				
Cognitive Area	N	Mean	SD	N	Mean	SD	Tests	FValue
Working Memory							M	8.07***
Digit Span†	68	3.12	0.37	75	3.33	0.41	A	9.92**
Digit Span Backward†	68	2.18	0.26	75	2.29	0.29	A	5.82*
Letter-Number (LNS)	68	9.57	2.74	75	11.73	2.39	A	26.57***
Arithmetic	68	11.59	2.83	75	11.48	2.89	A	0.006
Visual Memory							M	6.41***
Visual immediate	70	91.70	16.09	77	98.84	16.71	A	7.00**
Visual delayed	70	92.49	15.19	77	101.34	14.45	A	12.84***
Auditory memory							M	3.56**
Auditory immediate	70	100.13	15.81	78	107.32	13.69	A	9.66**
Auditory delayed	70	100.69	14.54	78	107.23	13.98	A	9.85***
Auditory recognition	70	102.64	15.94	78	106.03	13.30	A	2.41
Response speed								
Digit Symbol †	71	2.92	0.45	75	3.27	0.33	A	29.82***
Strategy Making								
Verbal fluency	71	33.77	12.01	77	44.21	10.47	A	30.23***

*** p<.001; ** p<.01; * p<.05; † transformed scores reported
A=ANOVA, M=MANOVA

Chapter 8 Further analyses: Exploring the control group cohort

8.1 Could the pattern of deficit observed in the initial analyses be due to selection of an inappropriate control group (i.e. rural police workers) who differ from farmers in some important way other than exposure history?

The previous chapters sought to establish whether the cognitive deficits observed in the farming cohort in Chapter 6 may have been due to an intrinsic problem with the exposed participants. Specifically, (1) whether they had mood disorder which could explain the pattern, or (2) whether the pattern was due to the accidental inclusion of acutely exposed participants. The results from both indicated that this was not the case. There is, however, another issue that needs consideration when evaluating the outcome of Chapter 6. Is it possible that the observed pattern was due to the use of an inappropriate control group who differ from farmers in some important way other than exposure history, resulting in exaggerated group differences?

A potential weakness of this study design which could limit the conclusions that can be drawn from the above analyses was the recruitment of rural police workers as an unexposed control group. Although matched to the farmers as far as possible in terms of characteristics which may affect cognitive function (i.e. age, gender, education level, premorbid IQ), police workers differ from farmers in terms of the exact nature of the work they undertake, lifestyle and life experiences. Differences in performance on neuropsychological testing between exposed farmers and unexposed rural police workers could be due to an unidentified confounder that was not controlled for in this study and may not reflect exposure history. Therefore, the above analyses were repeated using normative comparison standards.

8.2 Exposed cohort versus normative comparison standards: Re-analysis of the data using an alternative comparison group.

The neuropsychological test battery used in Chapter 6 consisted of well known, reliable and clinically sensitive measures for which population test norms are available. For example, the Wechsler Adult Intelligence scale and the Wechsler Memory Scale (Wechsler scales) have been developed over many years and the current editions are the result of extensive empirical studies in the US and UK involving a standardisation

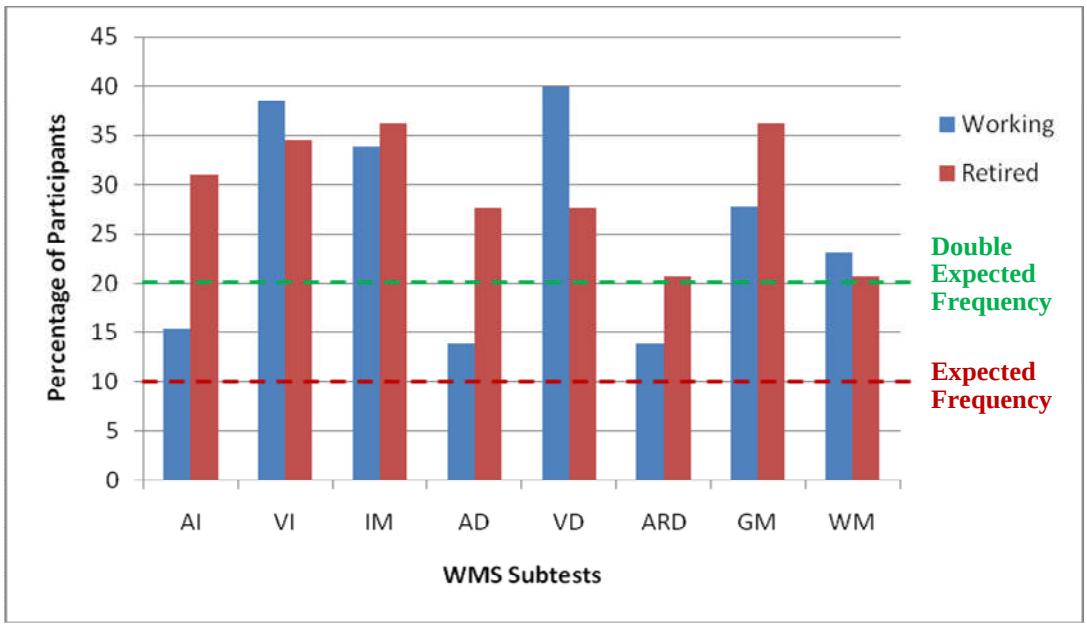
sample of over 2000 adults aged 16-90 years. The sample was divided into 13 age groups and stratified on key demographic variables including age, sex, years in education, race/ethnicity, geographic region. Extensive testing of reliability and validity were undertaken, including validation studies on clinical populations (learning disability, cortical and subcortical dementias, traumatic brain injury, multiple sclerosis, epilepsy, alcohol abuse, schizophrenia). The Wechsler scales provide contemporary normative information and interpretive tables allowing an individual's performance on these scales to be compared to national norms. Test norms were also available for all other measures included in our battery.

To determine whether organophosphates have a negative effect on cognitive function, the pattern of performance of the exposed group was compared to what one would expect to see in the normal population.

8.2.1 Wechsler Memory Scale – III

The Wechsler Memory Scale – III (WMS-III) was used to assess working, visual and auditory memory. Discrepancies between Intelligence (IQ) and memory are sometimes used to evaluate memory functioning. IQ score can be used as an index of probable, premorbid level of memory ability. Discrepancy scores between the IQ estimated memory performance and actual memory performance were calculated to indicate whether the exposed participants ability to learn and recall information was consistent with what would be expected given their intellectual functioning. The WMS-III manual provides tables which show whether a given discrepancy is statistically significant and what percentage of the standardisation sample had a discrepancy of that magnitude.

Figure 8.1 shows the percentage of people performing on the WMS-III sub-tests at a level seen in less than 10% of the standardisation sample. As can be seen in the graph below, the exposed cohort were more than twice as likely to perform poorly on tests of visual, working and general memory than the standardization sample. Retired farmers were more likely to likely to perform poorly on tests of auditory memory, in addition to the above.



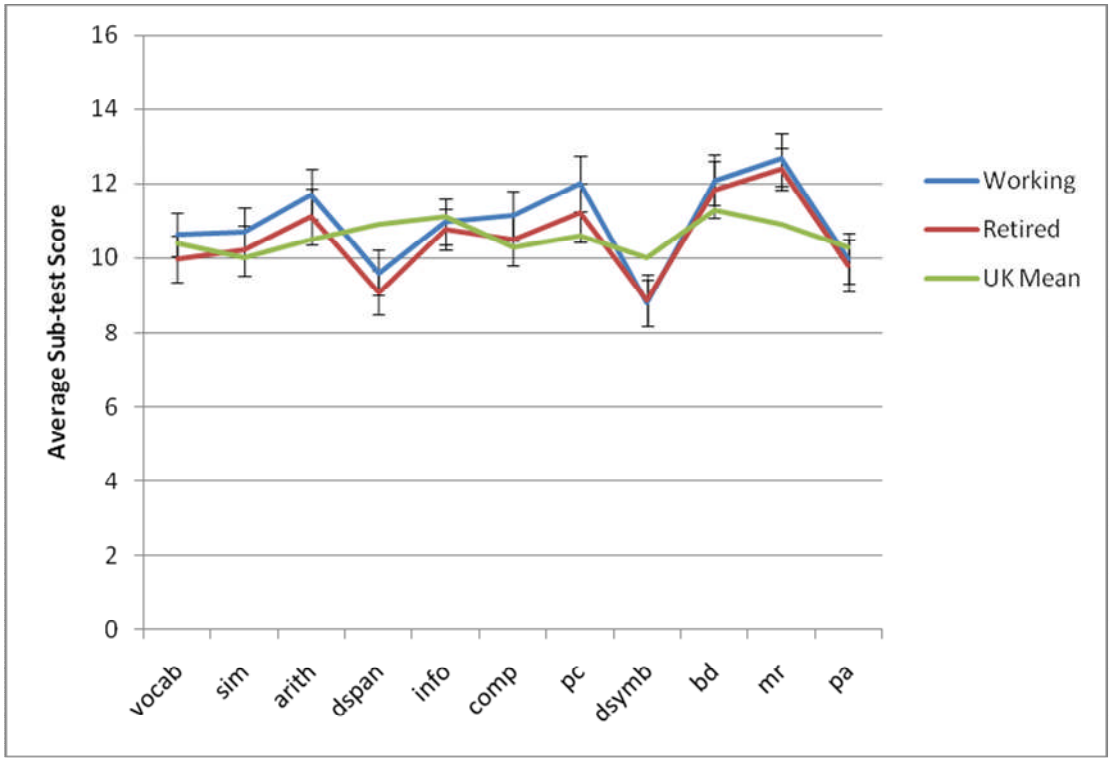
AI = auditory immediate memory; VI = visual immediate memory; IM = immediate memory score (aggregate of previous variables); AD = auditory delayed memory; VD = visual delayed memory; ARD = auditory delayed recognition memory; GM = general memory (aggregate of all scores); WM = working memory.

Figure 8.1 Percentage of people performing on the WMS-III sub-tests at a level seen in less than 10% of the standardisation sample.

A series of binomial tests with .1 set as the proportion of expected impairment revealed that significantly more working farmers were impaired than one would have expected on measures of visual, immediate and general memory (VI, VD, IM, GM; $p<.001$ for all) and working memory (WM; $p<.01$). Retired farmers were shown to be significantly impaired on all measures (ARD and WM; $p=.01$, all other measures $p<.001$).

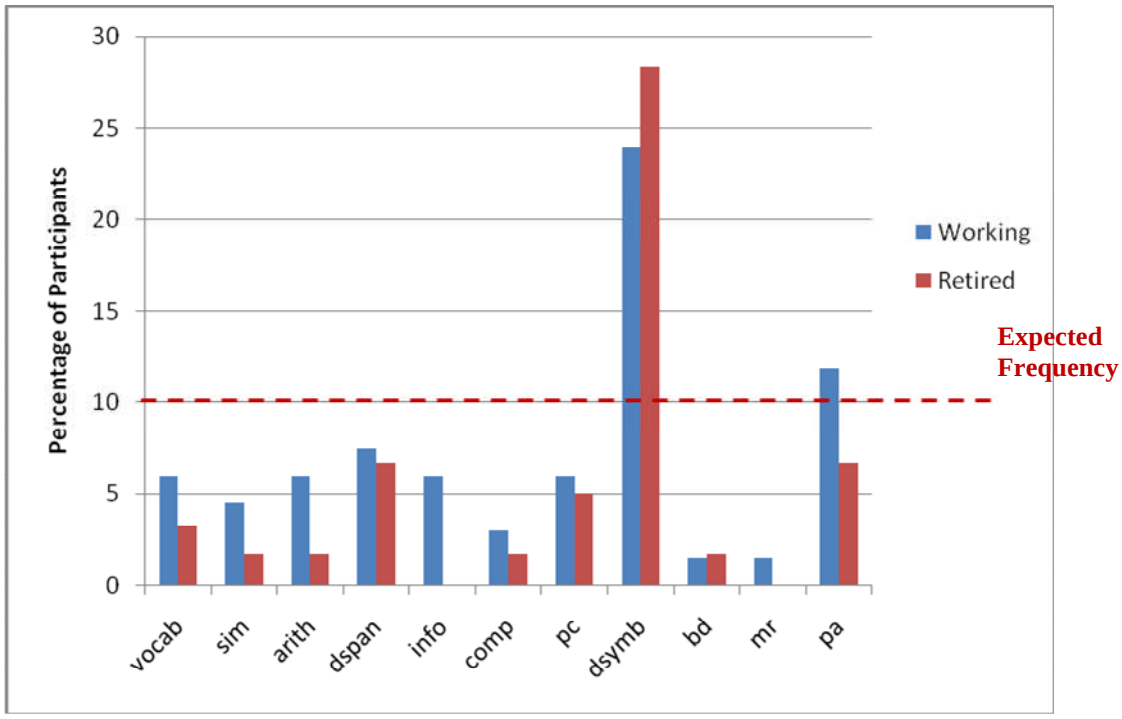
8.2.2 Wechsler Adult Intelligence Scale-III

The Wechsler Adult Intelligence Scale-III (WAIS-III) was administered to assess participants' current intellectual functioning. The test is comprised of 11 subtests which measure a range of cognitive functions such as working memory, response speed, verbal ability, verbal and visual reasoning and visuo-spatial ability. Figure 8.2 depicts the pattern of performance of study participants on the different WAIS-III subtests and Figure 8.3 shows the percentage of people in each group with significant impairment on WAIS sub-tests according to published test norms.



Vocab = vocabulary subtest; sim = similarities; arith = arithmetic; dspan = digit span; info = information; comp = comprehension; pc = picture completion; dsymb = digit symbol; bd = block design; mr = matrix reasoning; pa = picture arrangement.

Figure 8.2 Performance profiles on WAIS-III sub-tests (error bars represent ±2 S.E.), UK data taken from Wycherley, Lavender, Holttum, Crawford and Mockler (2005).



Vocab = vocabulary subtest; sim = similarities; arith = arithmetic; dspan = digit span; info = information; comp = comprehension; pc = picture completion; dsymb = digit symbol; bd = block design; mr = matrix reasoning; pa = picture arrangement.

Figure 8.3 Percentage of people significantly underperforming on the WAIS-III sub-tests.

From looking at Figures 8.2 and 8.3 it appears that overall the exposed cohort performed relatively similarly to that seen in the UK population. However, they appeared to show weaker performance on Digit Span (working memory) and Digit Symbol (response speed) subtests. This was true for both working and retired farmers. These findings were further investigated in terms of what one would expect to see in the standardization sample. Again, looking at impairment levels one would only expect to see in 10% of the standardization sample, a series of binomial tests with .1 set as the proportion of expected impairment were carried out. Results revealed that the only measure on which significantly more participants performed below that seen in the general population was on Digit Symbol; and this was true for both the working and retired cohort ($p<.001$ for both).

8.2.3 Additional tests

In addition to the abovementioned tests, further measures of response speed and executive function were collected (Trails A & B, verbal fluency, Stroop). Figure 8.4 shows the proportion of people in this study who performed with levels of impairment

one would only expect to see in 20% of the general population. A cut-off of 20% was selected because the norms for one of the tests (Trail Making A&B) indicated if individuals performed below the 20th percentile but did not provide any further information.

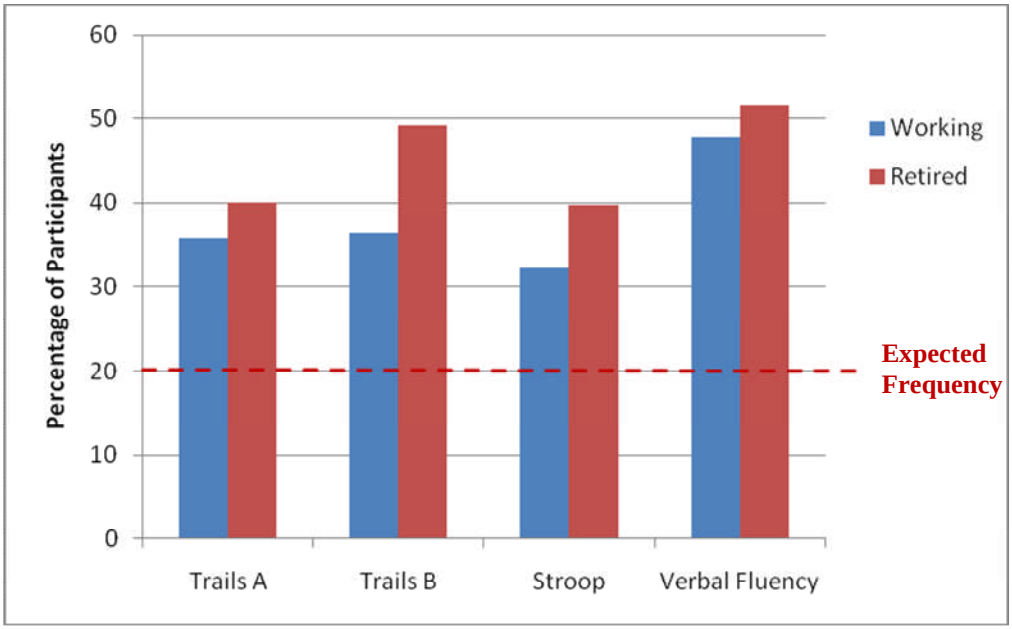


Figure 8.4 Percentage of people performing at a level seen in less than 20% of the standardisation sample on measures of executive function.

A series of binomial tests with .2 set as the proportion of expected impairment were carried out and revealed that both the working and retired farmers were significantly overrepresented at this level of impairment on all four measures (highest p=.01). This indicates that the OP exposed cohort performed more poorly on measures of executive function in comparison to the general population.

In addition, participants' performance of CALCAP simple & choice were analysed in terms of the frequency of participants who performed less than two standard deviations below normal. In a normal population we know that approximately 95% of the population falls between ±2 standard deviations from the mean. As such, we would expect to see about 2.5% of the population performing at a level of 2 standard deviations below the mean. Figure 8.5 shows the proportion of people in this study that performed at this level of impairment.

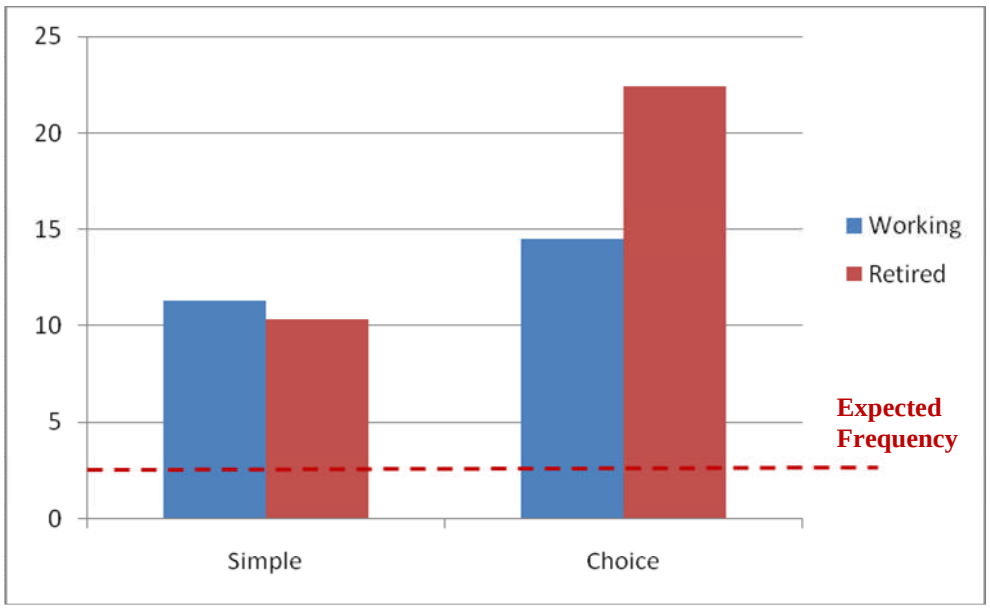


Figure 8.5 Percentage of people performing at an impairment level only expected in 2.5% of the population, on reaction time measures.

A series of binomial tests with .025 set as the proportion of expected impairment were carried out and revealed that both the working and retired farmers were significantly overrepresented at this level of impairment on both CALCAP measures (highest p=.003). This indicates that the OP exposed cohort showed significant impairments on measures of response speed and mental flexibility in comparison to the general population.

8.3 Summary & conclusions

In summary the findings above demonstrate that the exposed farmers showed significant underfunctioning in some cognitive domains, summarised in Table 8.1. The overall findings suggest that exposed participants have a consistent pattern of deficit, whether compared to a control group (in this case, rural police workers) or in comparison to published test norms derived from a cross section of healthy adults in the general population. Furthermore, the findings are consistent with the study hypotheses and show deficits on tests of working and general memory, response speed and mental flexibility, but preserved verbal, visuo-spatial, reasoning and general intellectual functioning.

Table 8.1 Areas of cognitive deficit in the exposed cohort.

	Working	Retired
General Intellectual Ability		
Response Speed	x	x
Working Memory*	x	x
Visual Memory	x	x
Auditory Memory		x
Verbal Abilities		
Mental Flexibility & Inhibition	x	x
Strategy Making	x	x
Verbal & Visual Reasoning Ability		
Visio-Spatial Abilities		

x = cognitive deficit present
* – Working memory is impaired according to performance on the WMS-III

Chapter 9 : The issue of exposure history; descriptive information and the relationship between cognitive function and indices of exposure.

9.1 Exposure history

9.1.1 Frequency and duration of exposure

Participants in this study had been exposed to OPs over a number of years as a result of sheep dipping and 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. Participants used a variety of OP products of differing compositions, most individuals using more than one product during their lifetime. Exposure history varied considerably despite participants appearing to have similar jobs (see Table and Figure 9.1.).

The average number of years farmers spent dipping sheep was 24.46 years (SD= 13) and farmers typically dipped twice a year for one day and for an average of about 7 hours per day. The mean time since farmers were last involved in dipping sheep was 10.31 years ago. No significant overall differences were observed between working and retired farmers in terms of these exposure variables.

9.1.2 Variability in the data

It is important to note that although no gross differences were found between the working and retired farming cohorts, there is a degree of variability amongst individuals in terms of their exposure history and this is apparent from looking at the large standard deviations relative to means, broad ranges and differences between the mean and modes and medians for some of the variables. Further exploration of the raw data was undertaken to determine whether there were outliers who may be biasing the mean and inflating the standard deviations.

Table 9.1 Duration and frequency of exposure to sheep dip and flock size (sometimes used as a proxy measure of exposure intensity).

Exposure variable	Mean	Standard deviation	Range	Median	Mode	Mann Whitney U
Years spent working with OPs						
Working Group	23.05	10.72	8-49	20	10	U=1737.5
Retired Group	26.11	15.17	5-66	24	7	p<.466
Total	24.46	13.01	5-66	22	10	
How many times per year						
Working Group	2.14	1.78	1-15	2	2	U=1825
Retired Group	2.03	1.38	1-10	2	2	p<.708
Total	2.09	1.58	1-15	2	2	
No. days per year						
Working Group	3.77	6.89	0.50-30	1	1	U=1674
Retired Group	2.84	3.54	1-21	2	1	p<.523
Total	3.34	5.61	0.50-30	1.75	1	
Hours per day						
Working Group	6.46	2.44	1.5-13	6	6	U=1568
Retired Group	6.69	2.47	2-12	7	8	p<.381
Total	6.57	2.45	1.5-13	7	8	
Years since last dipped						
Working Group	9.48	8.78	0-37	7.5	2	U=1568
Retired Group	11.25	7.65	0-42	10.5	6	p<.083
Total	10.31	8.2	0-42	9.5	2	
Flock Size						
Working Group	791.46	734.96	5-3,500	500	300	U=1882
Retired Group	1426.86	3065.35	3-20,000	500	400	P<.988
Total				500	300	

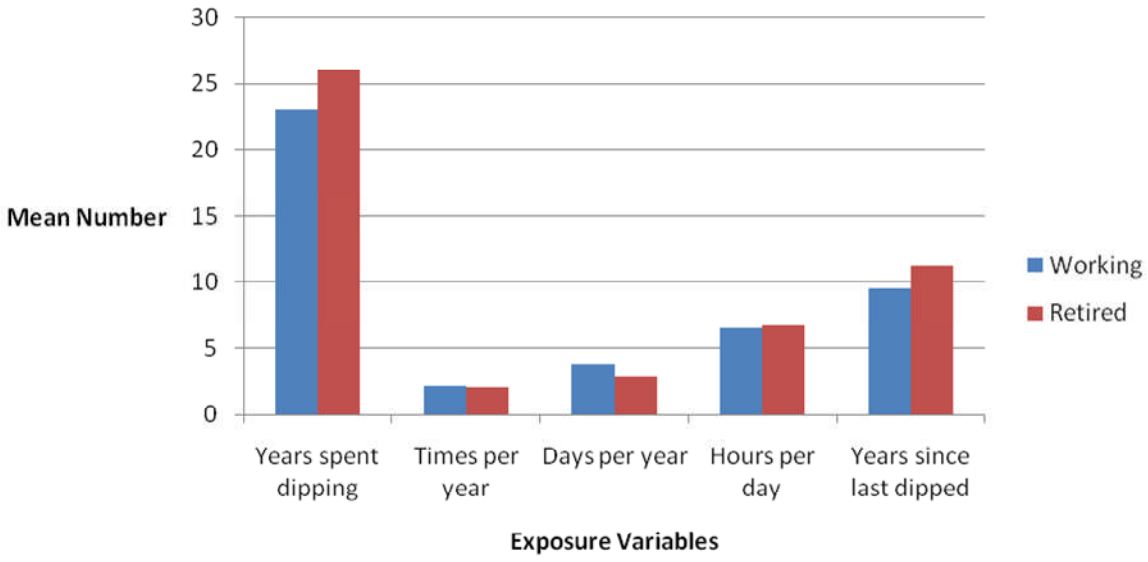


Figure 9.1 Exposure history: time spent dipping sheep.

No data entry errors were detected, but boxplots revealed a number of individuals whose exposure history deviated from the normal pattern, though not necessarily on all of the exposure variables simultaneously. For example, there were six individuals who dipped sheep for between 15-30 days per year as opposed to the more usual 1-2 days per year, yet in terms of frequency per year, they were not unusual in that five of them dipped twice a year. Had data only been collected on the number of times per year they dipped sheep, their increased intensity of exposure (relative to the majority of the cohort) would not have been detected. Three individuals dipped sheep between 7-15 times per year, rather than the more typical twice a year; and six individuals had very large flock sizes (between 2000 and 20,000). Flock size is sometimes considered a proxy measure of exposure intensity, since it takes longer to dip large flocks of sheep. These findings illustrate the importance of measuring many different aspects of exposure since variables such as duration, frequency and intensity of exposure may not always correlate. An individual may have a low frequency of exposure, but a high intensity of exposure or vice versa, according to these findings.

9.1.3 Other exposure variables – intensity of exposure

Measures such as years spent dipping, frequency, days per year, etcetera, give an indication of duration of exposure, but they do not necessarily provide a valid assessment of intensity of exposure. Therefore more detailed information about work

practices such as the primary task being performed, were collected as these variables can affect the intensity of exposure. Farmers were asked to specify the primary role they undertook whilst dipping and the results were as follows; 44% were responsible for 'dunking' sheep, which meant they stood by the dip bath and plunged each sheep under the surface with an implement or with their feet/hands. Dunkers are frequently splashed and soaked by sheep dip during the course of their work. 26% of farmers were 'chuckers' which meant they herded sheep in and out of the dipping facilities; and a tiny minority (3%) were 'helpers' who rounded up sheep prior to dipping.

Previous hygiene studies of sheep dipping in the UK have shown that the principle source of exposure to OPs is via the handling of concentrate dip and exposure to dilute dip wash through splashing (Buchanan et al, 2001). Hence, farmers were asked if they worked with concentrate and how often they were soaked with sheep dip during the dipping process. The majority of working and retired farmers worked with concentrate sheep dip (78% and 68% farmers respectively) and about half the cohort were regularly soaked by sheep dip during the dipping process (47% working and 58% retired farmers).

9.1.4 Dippers flu

Farmers were also asked whether they had experienced symptoms of 'dippers flu', a general malaise (headaches, aching limbs, runny nose, nausea, diarrhoea) that may occur after dipping, which may be indicative of undiagnosed acute toxicity (as discussed in Chapter 7). A total of 40% of farmers reported a history of 'dippers flu' (48% retired and 33% of working).

9.1.5 Exposure metrics

Accurate estimation of exposure is critical for the validity of studies investigating the adverse effects of exposure to pesticides and a number of different exposure metrics have been proposed by researchers in an attempt to consider all of the different aspects of exposure history which may be relevant. Exposure metrics vary greatly in terms of which variables they consider important and range from simple methods whereby different measures of frequency and duration of exposure are multiplied together to provide an overall rating; to complex formulae which attempt to estimate intensity of exposure, in addition to duration, by incorporating weightings for variables such as job

activity, use of protective clothing and so on. Exposure metrics do not reflect absolute exposure but provide a ranking within a population under study.

Two metrics were calculated in this study: a relatively simple estimate of 'lifetime exposure' based on the number of days per year spent using OPs multiplied by the number of years spent using OPs; and a more complex formula which took account of intensity in addition to duration of exposure ('ESK Metric'). This metric was originally constructed by Cherrie and Robertson (1995) and involves estimating the concentration of exposure through the skin (Csk) and area of skin contaminated (Ssk) according to job title and multiplying this information together with the cumulative amount of time the participants were involved in the dipping activity measured in terms of 8 hour days (t) in other words Esk = Csk x t x Ssk. Farmers exposure history profiles, estimated by these two metrics appear in Table 9.2.

Table 9.2 Exposure metrics - descriptive information.

EXPOSURE METRICS	Mean	Standard deviation	Range	Median	Mode	Mann Whitney
Lifetime index						
Working Group	87.92	177.39	8-1020	35	10	U = 1581
Retired Group	72.18	86.29	5-504	46.5	52	p=.35
Total	80.78	143.09	5-1020	36	10	
ESK index						
Working Group	136.08	477.26	0.38-2906.25	25.32	2.81	U=1534
Retired Group	68.63	85.72	0.33-405	36.45	16.20	p=.47
Total	105.85	359.41	0.33-2906.25	29.03	6.75	

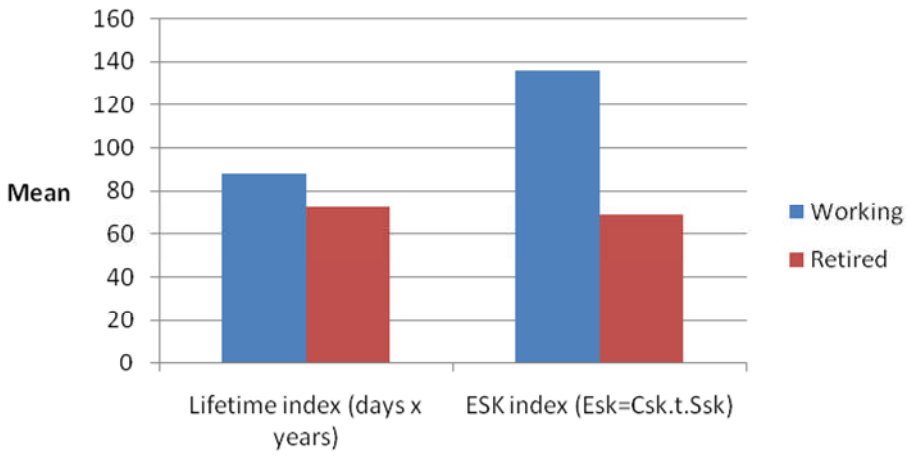


Figure 9.2 Exposure history: exposure metrics.

Again, working and retired cohorts do not appear to differ significantly in terms of these exposure metrics, but it is important to note the large standard deviations associated with these indices. The standard deviation indicates how well the mean represents the data and large standard deviations, relative to the mean, indicate that the data points are distant from the mean (Field, 2000). In other words there is a considerable amount of variability in this data. Furthermore, Figure 9.2 shows that the different metrics produce very different means for the working cohort, while producing similar indices for the retired, raising questions about the validity of these metrics.

9.2 Relationship between cognitive tests and exposure indices

In Chapter 6, the OP exposed cohort were found to perform significantly worse than healthy controls on a range of psychometric tests. Thus, operationalising exposure into "exposed" and "unexposed" groups yielded significant results. However, in this chapter analyses are undertaken, using Spearman correlations, to determine whether there is a linear relationship between indices of exposure and cognitive function *within* the exposed cohort.

To avoid Type I error the number of variables entered into the correlation matrix were limited. Instead of entering scores from all of the individual subtests included in the test battery, composite z-scores were calculated so that participant's performance in the 10 cognitive domains described in Chapter 6 could be examined in relation to exposure history. To do this, z-scores were created for each test score so that higher scores were interpreted as better performance (z-scores = (raw score – mean score)/stdev). To do

this reaction time date was reversed (Trails A and B, Grooved Pegboard and Stroop). Average z-score performance was then calculated for each domain. To avoid bias and determine the false discovery rate, all 10 cognitive domains were entered into the analyses and not just the domains shown to be impaired in the group analyses.

Table 9.3 Non parametric correlations between indices of exposure and measures of cognitive function.

	Yrs_dip	Lifetime_Exp	ESK	Age
	n=123	n=119	n=116	n=127
Working Memory	0.00	-0.07	0.00	0.11
Visual Memory	-0.24**	-0.14	-0.09	-0.07
Auditory Memory	-0.15	-0.12	-0.06	0.09
Response Speed	-0.21*	-0.14	-0.12	-0.08
Verbal Ability	-0.25**	-0.15	-0.07	-0.01
Mental Flexibility Inhibition	-0.23**	-0.17	-0.12	-0.34**
Strategy Making	-0.18*	-0.09	-0.06	-0.04
Verbal Visual Reasoning	-0.19*	-0.11	0.02	-0.07
Visio-Spatial Ability	-0.17	-0.15	-0.09	-0.13
Fine Motor Control	0.14	0.11	0.14	0.38**

Asterisks denote 2-tailed significance: ** - p<.01; * - p<.05

Spearman's correlations revealed significant, negative correlations between duration of exposure and Visual Memory (r_s = -.24, p<.01), Response Speed (r_s = -.21, p<.05), Verbal Ability (r_s = -.25, p<.01), Mental flexibility (r_s = -.23, p<.01), Strategy Making (r_s = -.18, p=.05) and Verbal-Visual Reasoning (r_s = -.19, p<.05) indicating an association between duration of exposure and impairments in these areas. No significant correlations were found between the cognitive domains and other exposure metrics.

However it is of note that while these correlations were nominally significant, none of them survived a Larzelere and Mulaik correction (Howell, 1992). This correction is a stepped procedure designed to control for Type I errors in correlations. Stage one of this correction is identical to a standard Bonferroni correction (.05/*k*), where *k* represents the total number of comparisons that were carried out. The number of tests that are significant at this stage (s_1) are noted, and the procedure moves to the next step. At the second stage the alpha level (.05) is divided by a new denominator: k-s_1 (i.e. the total number of comparisons minus the number of those recorded as significant at stage

1). Again, the number of tests reaching significance are counted (s_2), and the procedure moves on. Next, alpha is divided by k-(s_1+s_2). In summary, the total number of comparisons minus those recorded as significant at any previous stage. The procedure stops at the stage where no significant outcomes are recorded.

However a debate exists in the literature concerning the need to make p-value adjustments when multiple outcome measures have been used (Feise, 2002; Moran, 2003). Some researchers recommend adjusting the p-values when multiple measures have been used to reduce the risk of finding spurious, false positive results which have occurred by chance. Other authors argue that adjustments such as Bonferroni are overly conservative and in reducing the chance of making a Type I error, the risk of Type II error is increased. These authors suggest study findings are interpreted within the context of study design, methodology and sample size rather than relying on overly conservative statistical methods (Feise, 2002; Moran, 2003). Austin Bradford-Hill, a medical statistician, devised criteria to assist researchers in determining whether significant results are due to real biological effects rather than random chance. The main principles set forward by Bradford-Hill which he deemed necessary to provide adequate evidence of causation are that (1) findings are consistent with previous research (2) results can be reproduced (3) any associations found are plausible in terms of being compatible with existing theories and understanding of underlying processes (4) there is a temporal and specific relationship between a putative cause and effect (Bradford-Hill, 1965). The issues surrounding statistical correction and the conditions needed to establish causal relationships will be addressed in more detail in Chapter 14, the discussion section of this thesis.

9.2.1 Partial correlations with age

Age correlates significantly with duration of exposure (r_s = 0.38, p<.01), but interestingly, not with either of the exposure metrics. Age is also known to correlate with cognitive function and in this cohort it correlated with mental flexibility and fine motor control (see Table 9.3). Therefore, the above analyses were repeated, utilising partial correlation, to control for the potentially confounding effects of age, but all nominally significant correlations were lost following this process.

However, it may not be appropriate to partial out the effects of age in this circumstance. Age is inextricably linked with duration of exposure; and scores on most psychometric

tests have been age corrected, the exceptions being tests of response speed. Returning to the above table, age only correlates significantly with cognitive domains that incorporate response speed tests. It does not correlate with memory and verbal and visuo-spatial ability and reasoning tests which correlated significantly with duration of exposure in the initial analyses. Attempting to remove the effect of age in the above correlational analyses may be tantamount to removing the effect of exposure.

9.2.2 Possible contribution of undiagnosed acute toxicity

To examine the possible contribution of undiagnosed acute toxicity on the relationship between duration of exposure and cognitive function, farmers with dippers flu were examined as a separate group (N=51). It was possible to classify the frequency with which they suffered from dippers flu as follows: every time they dipped sheep, most times, sometimes, not often, once or twice after dipping sheep.

Correlations were run between frequency of dippers flu and the 10 cognitive domains. The following table depicts the 2-tailed Spearman correlation coefficients. Lower performance on tests of Strategy Making were associated with increased instances of dippers flu. However, while nominally significant, this finding did not survive a Larzelere and Mulaik correction.

Table 9.4 Correlations between instances of dippers flu and the 10 cognitive domains.

Cognitive Domain	**r**
Working Memory	-0.17
Visual Memory	0.26
Auditory Memory	0.15
Response Speed	-0.11
Verbal Ability	-0.13
Mental Flexibility	-0.27
Strategy Making	-0.38*
Verbal Visual Reasoning	-0.16
Visio-Spatial Ability	-0.08
Fine Motor Control	0.25

Asterisks denote 2-tailed significance: * - $p<.05$

9.3 Summary

Participants in this study had been exposed to OPs through sheep dipping for an average of 24 years and farmers typically dipped once or twice a year for one to two days. However, there was a degree of variability amongst individuals in terms of their exposure history with some participants deviating from the normal pattern on one or more variables such as duration, frequency or intensity of exposure. Measures such as years spent dipping, frequency, days per year, give an indication of duration of exposure, but they do not necessarily provide a valid assessment of intensity of exposure, so more detailed information was collected about work practices (e.g. handling concentrate, role performed, instances of 'dippers flu') and metrics constructed in an attempt to consider all of the different aspects of exposure history which may be relevant. However, the exposure indexes produced variable results raising questions about their validity.

Statistical analyses were undertaken to determine whether a linear relationship exists between indices of exposure and cognitive function. A number of significant, negative correlations were found between duration of exposure (but not the exposure metrics) and visual memory, response speed, verbal ability, mental flexibility, strategy making and verbal-visual reasoning. However these were lost following statistical correction for Type I error and when the possible confounding effects of age and undiagnosed acute toxicity were controlled for. This can be interpreted in two ways (1) the cognitive deficits identified in study participants were not caused by exposure to OPs but by some other confounding factor; (2) the cognitive deficits identified in study participants were caused by exposure to OPs, but may have been lost for one (or more) of the following reasons:

- The association was small and eradicated following the application of overly conservative statistical methods. As mentioned previously, a debate exists in the literature concerning the need to make p-value adjustments when multiple outcome measures have been used, as this increases the risk of Type II error, particularly when subtle or rare effects are being investigated.

- The study did not include a sufficient number of participants to reliably detect small effect sizes (i.e. the study was under-powered). For example, power

analysis indicates a sample size of 193 would be necessary to detect a small (r=0.2) relationship between exposure and cognitive function (alpha=.05; power =.80).

- Accurate estimation of exposure was impossible and critical exposure data was not captured, rendering exposure measures invalid. As mentioned earlier, it can be hard to obtain accurate estimates of exposure history in some occupational settings.

- The relationship between exposure to OPs and neurobehavioural functioning is not linear. A number of researchers have questioned the assumption that dose-response relationships are always linear. 'U' shaped and inverted 'U' shaped curves have been identified and threshold effects below which health effects are not apparent, but above which symptoms develop have also been noted (Hartman, 1995; Peterson Myers, Zoeller, vom Saal, 2009). Furthermore, genetic differences between individuals in their capacity to detoxify and metabolise xenobiotics may render some individuals more susceptible to the effects of certain chemicals than others, thereby compounding any dose-response relationships which may exist.

Interpretation of the findings in this chapter and the issues raised above will be re-visited in Chapter 14; the discussion section of the thesis.

Chapter 10 : Psychiatric functioning and OP exposure

10.1 Introduction

A principle aim of this thesis is to investigate whether farm workers with a history of low level exposure to OPs (insufficient to cause acute intoxication) will show evidence of mood disorder, above that seen in a control population. Several measures of emotional well being were included in this study for different purposes. Self-rating scales such as the Hospital Anxiety and Depression Scale and the Beck anxiety and depression inventories were included to screen for clinically significant levels of anxiety and depression and measure severity of anxiety symptoms and depression. However, self-assessment scales are only valid for screening purposes and definitive diagnosis requires a comprehensive clinical examination, therefore, we also included a structured clinical interview (SCID; First et al, 1997) in our assessment process.

Structured interviews provide an important method of standardising evaluations and improving diagnostic reliability and validity. The SCID was chosen for the current study because it was developed to standardise DSM-IV evaluations of mental disorders. DSM-IV is a manual published by the American Psychiatric Association to assist clinicians in evaluating mental health disorders in both children and adults. The SCID aims to improve the reliability of DSM diagnoses and has been extensively validated over the last decade (Rogers, 2001). Study participants were evaluated for the following: Past major depressive episode, current major depressive episode, dysthymic disorder; generalised anxiety disorder, panic disorder, anxiety disorder or depression due to a general medical condition. With regard to the latter, DSM-IV requires the symptoms of anxiety or depression to be a direct physiological consequence of the general medical disorder. Anxiety and depression which has arisen as an emotional reaction to having a general medical disorder is not encompassed under this diagnostic heading. Findings are outlined in the following section.

10.2 Prevalence of mood disorder in the exposed vs control cohort

10.2.1 Current major depressive episode

Overall, 10.7% of farmers met DSM-IV criteria for a diagnosis of current major depressive episode compared to only 2.6% of controls and this difference was

significant ($\chi^2(1)=4.31$, $p<.05$). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=0.58$, *ns*; retired: $\chi^2(1)= 4.58$, $p<.05$).

10.2.2 Past major depressive episode

Overall, 39.7% of farmers met DSM-IV criteria for a diagnosis of past major depressive episode compared to only 17.1% of controls and this difference was significant ($\chi^2(1)=11.12$, $p<.001$). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=0.51$, *ns*; retired: $\chi^2(1)= 16.25$, $p<.0001$).

10.2.3 Dysthymic disorder

Only a small proportion of farmers and controls met DSM-IV criteria for a diagnosis of dysthymic disorder (4.9% and 2.6% respectively) and no significant difference was found between these two groups ($\chi^2(1)=0.63$, *ns*).

10.2.4 Depression due to a general medical condition

Only a small proportion of farmers and controls met DSM-IV criteria for a diagnosis of depression due to a general medical disorder (4.3% and 2.7% respectively) and no significant difference was found between these two groups ($\chi^2(1)=0.30$, *ns*).

10.2.5 Panic disorder

Overall, 9.1% of farmers met DSM-IV criteria for a diagnosis of panic disorder compared to only 1.3% of controls and this difference was significant ($\chi^2(1)=4.93$, $p<.05$). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=1.13$, *ns*; retired: $\chi^2(1)= 4.3$, $p<.05$).

10.2.6 Generalized anxiety disorder

Overall, 14.2% of farmers met DSM-IV criteria for a diagnosis of generalized anxiety disorder compared to only 1.3% of controls and this difference was significant ($\chi^2(1)=9.07$, $p<.005$). However, when this was broken down for the two working status groups, it was only found to be significant for the retired cohort (working: $\chi^2(1)=1.85$, *ns*; retired: $\chi^2(1)= 8.06$, $p<.005$).

10.2.7 Anxiety due to a general medical condition

Only a small proportion of farmers and controls met DSM-IV criteria for a diagnosis of anxiety due to a general medical disorder (0.9% and 1.4% respectively) and no significant difference was found between these two groups ($\chi^2(1)=0.094$, *ns*).

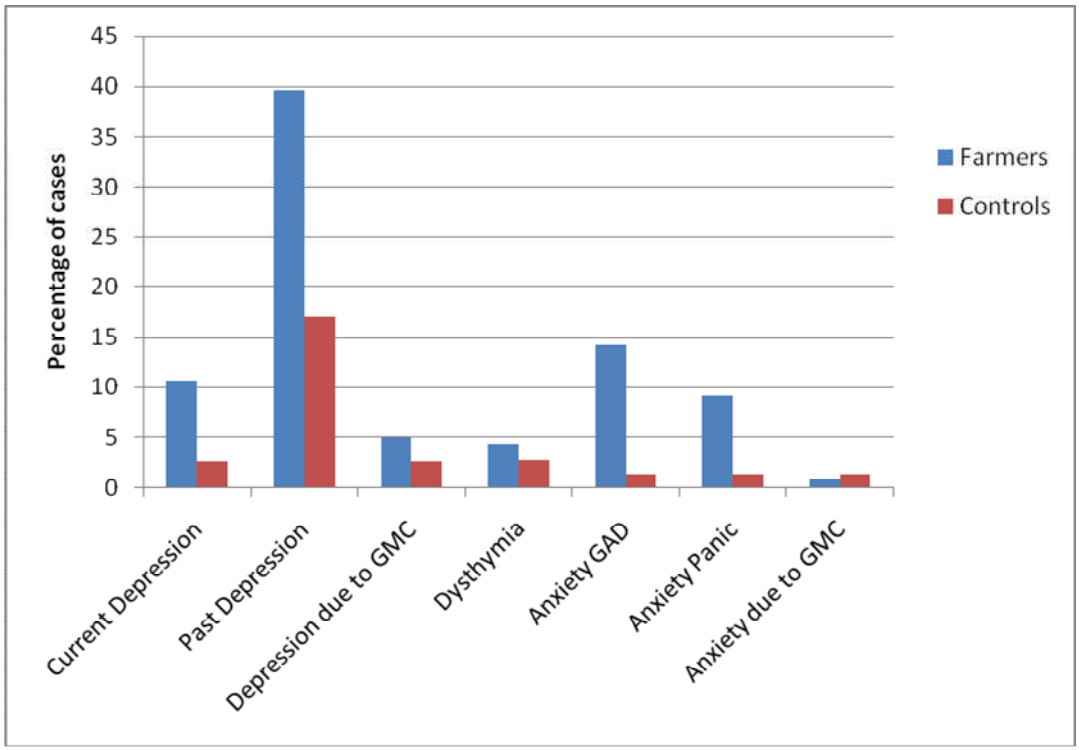


Figure 10.1 Percentage of farmers and controls who meet DSM-IV criteria for a diagnosis of anxiety and depression.

In summary, rates of depression and anxiety disorder were much higher amongst farmers than controls (see Figure 10.1). Few participants met criteria for anxiety or depression due to a general medical disorder, or suffered from dysthymia, so these diagnostic categories do not appear in subsequent tables and graphs.

Overall, these results concur with earlier findings using the Hospital Anxiety and Depression screening measure. However, it is important to note that a much smaller number of farmers and controls meet DSM-IV criteria for a diagnosis of anxiety or depression than the numbers found to be reporting significant levels of anxiety and depression on the self-report screening tool (see Table 10.1).

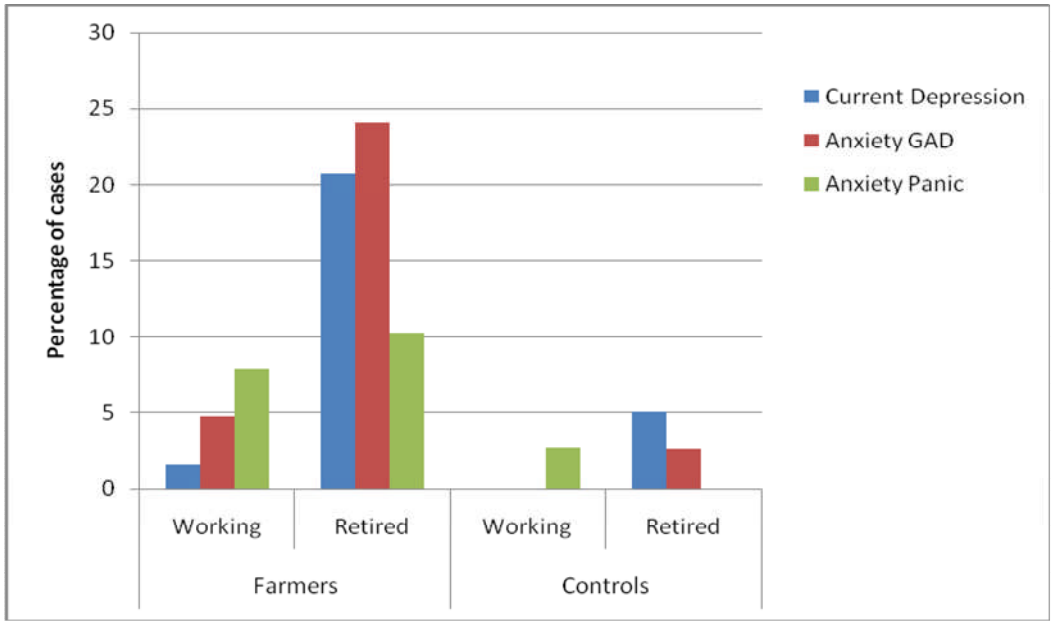
Table 10.1 Rates of anxiety and depression according to different methods of assessment; a self-report screening tool (HAD) and a structured clinical interview for DSM-IV disorders.

Disorder	HAD*	DSM-IV
Depression		
Farmers	45.9%	10%
Controls	6.6%	2.6%
Anxiety (GAD)		
Farmers	41%	21.7%
Controls	22.4%	2.7%

*Please note, the HAD does not split anxiety into generalised anxiety and panic disorder, so diagnoses were collapsed on the SCID to match.

Analyses concerning the rates of mood disorder and anxiety in the different working status groups reveals much higher rates of these conditions in retired participants, particularly in the farming cohort (see Figure 10.2).

Figure 10.2 Percentage of farmers and controls who meet DSM-IV criteria for a diagnosis of anxiety and depression, split by working group.



In terms of symptom severity, more farmers than controls describe their symptoms of anxiety and depression as being of moderate to severe severity (see Table 10.2).

Table 10.2 Percentage of participants with mood or anxiety-related disorders by severity.

	Anxiety		Depression	
	Exposed Group	Control Group	Exposed Group	Control Group
Normal	58.7	83.1	53.7	87.0
Mild	16.5	14.3	15.7	9.1
Moderate	19.0	1.3	17.4	3.9
Severe	5.8	1.3	9.9	0

10.2.8 Possible causation

An important issue is the extent to which the anxiety and depression seen in farmers is related to exposure to OPs (either directly as a result of damage to the central nervous system, or indirectly as an emotional reaction to more general ill health); or has arisen as a consequence of some other factor such as stressful life events or a reaction to retirement.

Given rates of anxiety and depression are higher amongst farmers who have retired on ill health grounds compared to controls who have retired on ill health grounds, it seems likely that an additional factor, other than retirement per se accounts for the levels of distress noted in farmers.

A life events checklist (Holmes and Rahe, 1967) was included in an attempt to tease apart the relative contribution of recent stressful life events and exposure to pesticides in triggering mood disorder. This scale consists of 43 positive and negative life events (such as divorce, marriage, retirement, change in financial state) capable of inducing stress and ill health. Each life event is assigned a value according to how stressful they were considered to be by a standardisation sample of 394 individuals. Participants in the current study were asked to state whether they had experienced any of these events over the last 12 months. Scores above 150 are associated with an increased risk of illness and a score above 300 is associated with a high risk of developing a stress related illness. Descriptive information is provided in Table 10.3 but it is important to note that the life event data was not normally distributed.

Table 10.3 Descriptive statistics for stressful live events scores for the exposure and non-exposed cohorts.

	Mean	Standard deviation	Range
Farmer			
Working	49.89	58.16	0-199
Retired	106.07	78.68	0-311
Control			
Working	73	69.37	0-254
Retired	96.21	69.60	0-286

Review of the descriptive data reveals that only a small proportion of working farmers and controls obtained a total score of over 149 on this measure (9.2% and 8.6% respectively) but around a fifth of retired farmers scored above this cut off (20.3% retired farmers vs 15.4% retired controls). No controls scored above the cut off of 300 on this measure, but 2% of retired farmers obtained high scores. However, statistical analysis indicated that the differences noted between farmers and controls in terms of the scores they obtained on the life events scale, were not significantly different (U= 4.157, *ns*). In other words, the rate of recent stressful life events is equivalent in the exposed farmers and unexposed controls.

Not surprisingly high scores on the life events scale are associated with increased severity of anxiety symptoms and mood disorder (see Table 10.4), but given that the rate of recent stressful life events is equivalent in the exposed farmers and unexposed controls, life stress per se cannot be the sole factor underlying the increased rates of psychiatric disturbance observed in the farming cohort.

Table 10.4 Correlations between self-report measures of anxiety and depression and stressful life events experienced over the last year.

	HAD Anxiety	HAD depression	Beck Anxiety	Beck Depression
Farmer Life events score	.280**	.250**	.310**	.320**
Control Life events score	.430**	.322**	.375**	.433**

**Significant at p<0.01 (2-tailed)

As the exposed cohort show a significantly higher incidence of mood disorder than controls, and given that the two groups do not differ in terms of stressful life events, it seems that OP exposure may indeed have a part to play in the depression and anxiety seen in the farming cohort.

In order to see whether exposure has a linear relationship with anxiety and depression, exposure variables (lifetime exposure index, Esk, duration of exposure (in years), years since last dip) were entered into partial correlations with HAD, BDI and BAI scores, while controlling for recent stressful life events. No significant results were found.

10.2.8 Summary

A principle aim of this study is to investigate whether farm workers with a history of low level exposure to OPs show evidence of mood disorder. Several measures of emotional well being were included in this study. Rates of depression and anxiety were much higher amongst farmers than controls, especially amongst retired farmers. Rates differ according to the method used to evaluate mood and a smaller percentage of farmers meet DSM-IV criteria for a diagnosis of anxiety or depression than the numbers who complain of significant levels of distress on self-report screening tools.

An important issue is the extent to which the mood disorder observed in farmers is due to exposure to OPs as opposed to lifestyle factors. Findings indicate that retirement per se is unlikely to be solely responsible for the mood disorder observed in retired farmers as controls who had also retired on ill health grounds had lower rates of mood disorder than farmers. Stressful life events, although associated with increased severity of mood disorder, are unlikely to be solely responsible for elevated rates of anxiety and depression in farmers as the rate of recent stressful life events is equivalent in the exposed farmers and unexposed controls.

However, no significant correlations were observed between indices of exposure to OPs and mood, possibly the study did not include a sufficient number of participants to reliably detect small effect sizes; or because exposure measures invalid; or the relationship between exposure to OPs and neurobehavioural functioning is not linear.

Alternatively, the mood disorder observed in farmers may not have been caused by exposure to OPs and could be due to some other factor. Individuals with a history of exposure to OPs often complain of chronic ill health and a constellation of physical

symptoms which can be severe and disabling (Mackenzie Ross et al 2007). The elevated rates of anxiety and depression observed in farmers who took part in this study, may have arisen as a reaction to deteriorating physical health and well being. In the next chapter, the physical health of farmers is examined and compared to that of unexposed controls and the possible relationship between symptoms of ill health and exposure to OPs is explored.

Chapter 11 : Physical Symptoms and OP exposure

11.1 Introduction

In Chapter 2, the toxicology of OPs and associated health effects were described in some detail. To summarise, organophosphates are readily absorbed by the body through the skin, lungs, gastrointestinal tract and conjunctiva. Exposure to OPs can result in acute and chronic health effects. The immediate effects of OP poisoning which occur within hours of exposure have been well documented (Baxter et al, 2000; COT Report, 1999; ECETOC Report, 1998; Royal Colleges' Report, 1998; WHO Report, 1990) and involve inhibition of the enzyme acetylcholinesterase, causing changes in peripheral, autonomic and central nervous system function (cholinergic crisis). Recovery from mild poisoning occurs rapidly (circa 24-48 hours) and 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 that have persisted long after resolution of the cholinergic crisis (COT Report; Royal Colleges Reports). OPs are capable of producing several delayed physical and neurological syndromes which are not well known or understood and may be unrelated to the cholinergic effects. These include *The Intermediate Syndrome* which involves proximal flaccid limb paralysis typically starting 1-4 days after poisoning and lasting 5-18 days; o*rganophosphate induced delayed polyneuropathy (OPIDN),* a delayed sensory and motor polyneuropathy affecting predominantly the lower limbs, but in severe cases the upper limbs as well. OPIDN onsets around 2-4 weeks after exposure and recovery is slow and often incomplete; and *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), hypoxic brain damage (Baze, 1993) and apopotic neuronal cell death (Abou-Donia, 2005; Kapur et al, 2007). IS and OPIDN usually follow an episode of acute poisoning but neurobehavioural deficits have been associated with both high and low-level exposure (Brown & Brix, 1998; Davies, Ahmed and Freer, 2000; Kamel and

Hoppin, 2004).

Previous studies of sheep farmers, regularly exposed to OPs, have found a high incidence of symptoms consistent with chronic fatigue syndrome such as extreme fatigue, impaired memory and concentration, aching joints, aching muscles, muscle weakness, headaches, sleep disturbance, irritability, word-finding difficulties, depression, anxiety, parasthesia, gastro-intestinal disturbance and respiratory disorders (Ahmed & Davies 1997; Beach et al, 1996; Davies et al, 1999; Dunn, 2002; Fletcher, et al, 2005; Jamal et al, 2002; Mackenzie Ross et al, 2007; Pilkington et al, 2001; Solomon et al, 2007; Tahmaz, Soutar and Cherrie, 2003).

Ahmed and Davies 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, although they did not provide details about the individuals' exposure histories, other than to state that many suffered episodes of dippers flu.

In 1999 Davies, Ahmed and Freer described the results of 2 postal surveys in which they attempted 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.

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 (Fletcher, MacLehose, Hurley et al, 2005).

In 2006 Solomon et al reported the findings from a postal survey of men born between 1933 and 1977 who were resident in three rural areas of England and Wales. Neuropsychiatric symptoms were more common in past users of sheep dip than in men who had never used pesticides; but symptoms were also common in men who had used pesticides other than sheep dip. Among users of sheep dip, prevalence was higher amongst men who dipped most often, but the authors suggest non- toxicological factors may account for these findings as they did not find an elevated risk of ill health in those individuals who handled sheep dip concentrate and because symptoms were reported by men who had used other pesticides. The authors suggest public concern about the adverse effects of pesticides may influence reporting and that individuals who have a tendency to dwell on somatic complaints are more likely to report ill health.

Mackenzie Ross et al (2007) compared 25 farm workers with a history of apparent low level exposure to sheep dip with 22 non-exposed healthy volunteers on

neuropsychological tests. All reported a range of physical symptoms the most prominent being fatigue, aching muscles and joints, headaches, sleep disturbance and irritability. Agricultural workers described their symptoms as being severe and disabling and two thirds of them had retired or reduced their workload on ill health grounds. All were involved in litigation and so it is unclear how representative they are of the farming community as a whole.

Although previous studies undertaken in the UK suggest a link between exposure to sheep dip and the development of neurobehavioral problems, it is unclear whether this is due to a history of acute poisoning or a result of cumulative low level exposure. In the present study, farmers and controls were asked to complete a questionnaire regarding their physical health. This provided two types of information (1) a generic measure of disease burden which was derived by asking participants to rate their health as being excellent, very good, good, fair or poor, and (2) more detailed information about health symptoms, their date of onset, frequency, severity and impact on every day functioning. The questionnaire was based on a number of existing questionnaires with known reliability and validity and additional symptoms, not associated with exposure to neurotoxic agents, were added to the list to determine whether farmers complain of symptoms associated with neurotoxic exposure or an array of symptoms with different underlying causes.

11.2 Incidence of physical health problems

Participants were given a list of 39 symptoms (see Appendix 2) and asked if they had experienced them in the last 4 weeks and if so, to rate their frequency and severity. The symptoms listed in the questionnaire potentially reflect central, peripheral and autonomic nervous system damage; but are also relatively non-specific and may have multiple aetiologies. In addition, participants were asked how much pain they were in on a scale of 0 (none) to 5 (extremely severe) and how much this impacted on their social life (this time on a 0-4 point scale from not at all to extremely).

The health symptoms reported by study participants are summarised in Table 11.1.

Table 11.1 Physical health ratings of study participants according to occupational group and working status.

	Working		Retired	
	Mean	Standard Deviation	Mean	Standard Deviation
Overall Health Rating				
Exposed Group	2.53	0.78	3.89	.99
Control Group	2.05	0.80	3.08	1.13
Number of Moderate-Severe Symptoms				
Exposed Group	2.90	3.34	10.76	7.14
Control Group	1.49	1.42	2.52	2.01
Pain				
Exposed Group	1.59	1.15	2.65	1.23
Control Group	1.32	1.12	2.39	0.96
Impact on Social Life				
Exposed Group	0.2	0.54	1.74	1.47
Control Group	0.16	0.69	0.69	1.14

As the assumptions of normality and homogeneity of variance were violated for all variables, a series of Mann Whitney U tests were used to investigate whether there were any significant differences in the mean overall health rating scores or the amount of moderate to severe physical symptoms the exposed and control groups reported having.

Overall, and in both the working and retired cohorts, the exposed participants reported significantly worse general health than the controls (U=3097.5, p<.001; U=829.0, p<.01; U=606.05, p<.001 respectively). A similar pattern was found in terms of the number of moderate to severe symptoms that were reported, with the exposed group reporting more symptoms than the controls in both the working (U=990.0, p<.05) and retired (U=292.5, p<.001) cohorts, as well as overall (U=1614.5, p<.001).

In contrast, there was no significant difference between the exposure groups in terms of reported pain for either the working or retired cohorts (U=1059, *ns*; U=823.5, *ns* respectively), nor overall (U=3957, *ns*). However, the exposed cohort reported their symptoms had a significantly greater impact on their social life (U=3632, p<.01) compared to controls, although this only held true for the retired cohort (retired: U=611.0, p=.001; working group: U=1185.5.00, *ns*).

Further Mann Whitney U tests revealed that the retired cohort reported significantly worse overall health (U=1937, p<.001) than the working group. The retired participants also reported suffering from a larger number of moderate to severe symptoms (U=2485.5, p<.001), feeling a greater amount of pain (U=2431.5, p<.001) and experiencing more interference from these symptoms with their social life (U=1403, p<.001). This pattern also held true in all cases when working status was looked at separately for the two exposure groups (U range: 243.5-962.5, largest p=.02).

11.2.1 Incidence of physical health problems and impact on mood

The previous chapter outlined greater problems with mood in the exposed cohort, compared to the control groups. In order to see whether the health variables described in the previous section may be related to mood, Spearman's correlations were carried out with HAD, BDI and BAI scores. The results in Table 11.2 show a strong relationship between mood and physical symptoms for both groups.

Table 11.2 Correlations between mood and physical health.

	Exposed Cohort			Control Group		
	Health rating	Pain rating	Social impact	Health rating	Pain rating	Social impact
BDI	0.45*	0.36*	0.53*	0.44*	0.32*	0.36*
BAI	0.31*	0.40*	0.43*	0.42*	0.27	0.29
HADSD	0.47*	0.36*	0.51*	0.51*	0.32*	0.40*
HADSA	0.35*	0.36*	0.36*	0.43*	0.35*	0.41*

-Asterisks denote significant correlations after L&M corrections have been applied

11.3 Pattern of physical health problems

In order to establish whether there were any specific symptoms that the exposed cohort was more likely to complain of compared to controls, symptom profiles were established for the two groups. To do this, the frequency with which participants complained of specific physical ailments as being moderate-to-severe was investigated (see Figure 11.1). Odds ratios were then calculated for each of the 39 physical symptoms by participants who were exposed to OPs and those who were not (these can be seen in Table 11.3). An odds ratio greater than 1 indicates that the condition is more likely to occur in the exposed cohort. In contrast, an odds ratio less than 1 indicates that the condition is more prevalent in the control group.

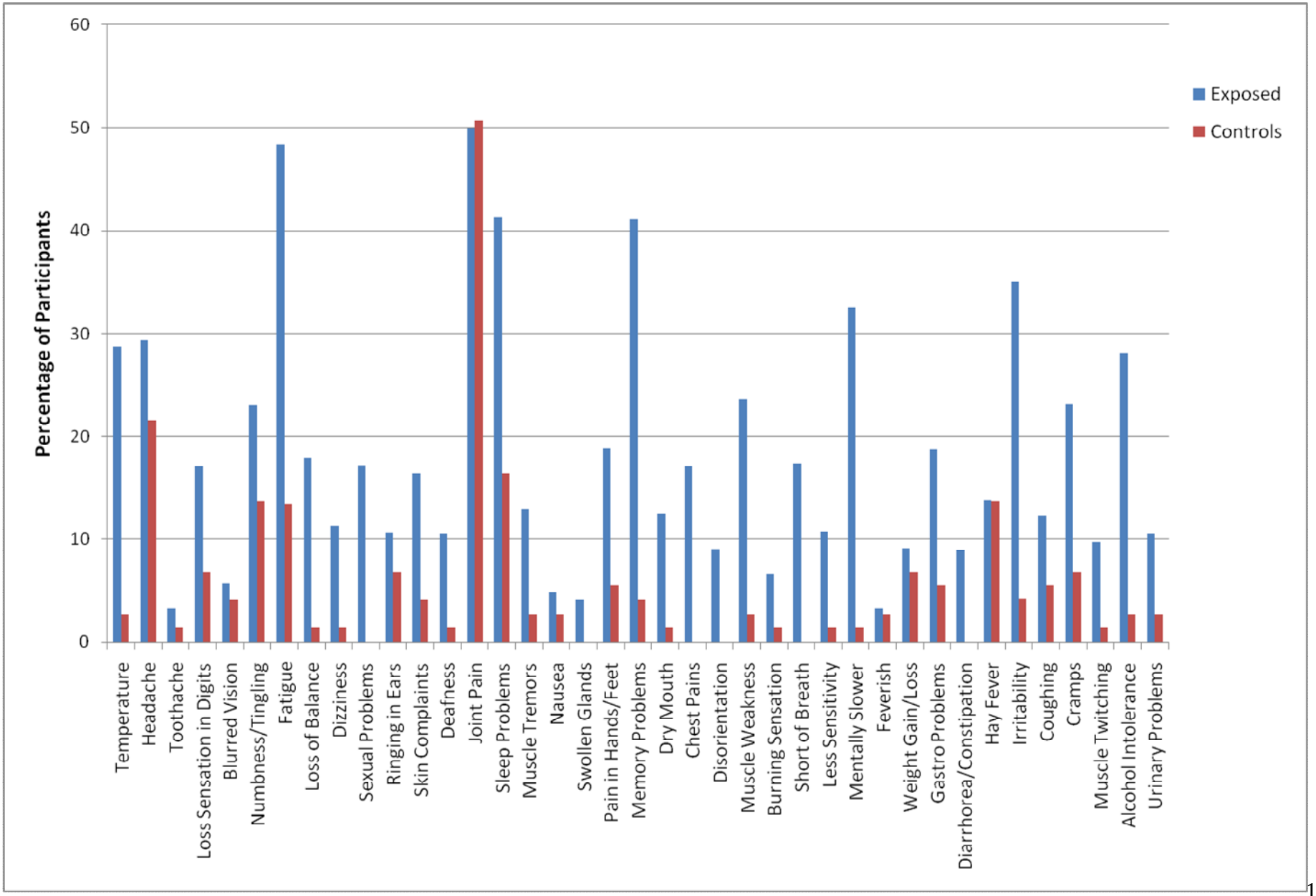


Figure 11.1 Frequency of moderate-severe physical symptoms.

Table 11.3 ODDs ratios for the physical symptoms.

	ODDS Ratio	Rank Order	New α	χ^2 significant
Mental Slowing	33.91	1	0.002	*
Memory Problems	16.32	2	0.002	*
Loss of Balance	15.36	3	0.002	*
Temperature	14.51	4	0.002	*
Alcohol Intolerance	14.08	5	0.002	*
Irritability	12.28	6	0.002	*
Muscle Weakness	11.13	7	0.002	*
Dry Mouth	10.06	8	0.002	ns
Dizziness	8.97	9	0.002	ns
Less Sensitivity	8.44	10	0.002	*
Deafness	8.26	11	0.002	ns
Muscle Twitching	7.57	12	0.002	ns
Fatigue	6.01	13	0.002	*
Muscle Tremors	5.34	14	0.003	ns
Burning Sensation	4.98	15	0.003	ns
Skin Complaints	4.59	16	0.003	ns
Urinary Problems	4.23	17	0.003	ns
Cramps	4.12	18	0.003	ns
Pain in Hands/Feet	3.98	19	0.003	ns
Gastro Problems	3.95	20	0.004	ns
Sleep Problems	3.59	21	0.004	*
Loss Sensation in Digits	2.83	22	0.004	ns
Coughing	2.41	23	0.005	ns
Toothache	2.40	24	0.005	ns
Numbness/Tingling	1.88	25	0.006	ns
Nausea	1.82	26	0.006	ns
Ringing in Ears	1.63	27	0.007	ns
Headache	1.50	28	0.008	ns
Blurred Vision	1.41	29	0.010	ns
Weight Gain/Loss	1.37	30	0.013	ns
Feverish	1.23	31	0.017	ns
Hay Fever	1.01	32	0.025	ns
Joint Pain	0.97	33	0.050	ns
Sexual Problems	undefined	unable to carry out chi square due to empty cells		
Chest Pains	undefined			
Disorientation	undefined			
Short of Breath	undefined			
Diarrhoea/Constipation	undefined			
Swollen Glands	undefined			

Chi Square tests were also carried out to detect differences in the frequency of these physical complaints between the two groups and Bonferroni-Holms tests applied to control for Type I error. This involved deriving revised alpha levels by using the formula:

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

where $\alpha^{\text{original}} = .05$; 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).

The results, which can be seen in Table 11.3, highlight 10 physical symptoms which readily distinguish the exposed and control groups. These can also be seen in Table 11.4, where they are classified by symptom type. From viewing the odds ratios, we can see that for each of these 10 symptoms farmers are between 3.59 and 33.91 times more likely to be symptomatic than the controls. It is interesting to note that the majority of these symptoms can be described as central nervous system (CNS) problems, and half of them are of a psychological nature. Symptom reporting for musculo-skeletal or dummy variables did not distinguish the exposed group from the controls. This suggests the farmers complain of specific symptoms and are not simply endorsing a broad array of non-specific symptoms.

Table 11.4 Classification of symptom type.

Symptom	Problem Type
Mental Slowing	CNS
Memory Problems	CNS
Loss of Balance	CNS
Temperature	ANS
Alcohol Intolerance	CNS
Irritability	CNS
Muscle Weakness	PNS
Reduced Sensitivity	PNS
Fatigue	CNS
Sleep Problems	CNS

11.4 Physical health and exposure

When operationalising exposure with the farmers and controls, the exposed cohort show a specific pattern of physical health problems compared to the controls. In order to see

whether exposure has a linear relationship with physical health problems, the exposure indices (lifetime exposure index, Esk, duration of exposure, years since last dip) were correlated with the frequency (ranging from 0 meaning "never" to 3 representing "all the time") with which participants reported experiencing each of the aforementioned 10 symptoms. The number of these 10 symptoms each participant reported was also entered into these Spearman correlations. No significant results were obtained.

However, while no significant correlations were found between physical health and exposure, that does not mean that there is no relationship between the variables; just that it may not be a linear one. To investigate this another way, odds ratios were calculated for each of the 10 physical symptoms for exposed participants who were in the highest quintile of the different exposure indices, versus those who fell into the other four quintiles (these can be seen in Table 11.5). An odds ratio greater than 1 indicates that the condition is more likely to occur in the highest exposed cohort. In contrast, an odds ratio less than 1 indicates that the opposite is true.

Table 11.5 ODDs ratios for the difference exposure metrics.

	ODDs Ratios		
	Lifetime Exposure	EsK Index	Duration of Exposure
Mentally Slower	1.50	1.53	1.01
Memory Problems	0.85	1.43	1.51
Loss of Balance	3.07	1.79	1.81
Temperature	1.37	1.23	2.04
Alcohol Intolerance	1.17	0.52	0.18
Irritability	1.26	1.73	1.58
Muscle Weakness	1.07	1.14	1.96
Less Sensitivity	3.19	2.16	0.70
Fatigue	1.72	1.65	2.36
Sleep Problems	0.96	1.40	1.09

These findings illustrate that in the majority of cases, the odds of a participant being symptomatic are higher for those in the highest exposure quintile than those who are not. This suggests that exposure may indeed have a role to play in physical health, although the precise relationship between these variables is unclear and warrants further investigation.

11.5 Summary

Exposure to OPs can result in acute and chronic health effects and previous studies of sheep farmers have found a high incidence of symptoms consistent with chronic fatigue syndrome such as extreme fatigue, impaired memory and concentration, aching joints, aching muscles, muscle weakness, headaches, sleep disturbance, irritability, word-finding difficulties, depression, anxiety, parasthesia, gastro-intestinal disturbance and respiratory disorders.

Farmers who took part in the present study reported significantly worse general health than controls and an increased number of moderate to severe symptoms. The ten physical symptoms which readily distinguished the exposed and control groups were consistent with those reported in earlier studies, but the frequency with which they suffered from these symptoms did not correlate significantly with indices of exposure. However, the odds of a participant being symptomatic are higher for those in the highest exposure quintile than those who are not and this suggests OP exposure may indeed have a role to play in physical health, although the relationship may not be linear. Analyses also revealed a strong relationship between depression and health ratings indicating farmers experience these symptoms as distressing and disabling.

Chapter 12 : Potential susceptibility to OPs - Genetic data

12.1 Genes and OPs

Paraoxonase (PON1) is a liver and plasma enzyme which may protect against cardiovascular disease because it metabolises oxidized lipids, thus preventing the accumulation of lipoprotein on the arterial wall. It also plays a role in the detoxification of organophosphate pesticides (OPs) and received its name from its capacity to hydrolyse paraoxon, a toxic metabolite of the insecticide parathion. PON1's capacity to metabolise and detoxify OPs makes it an important consideration for any study seeking to address the health effects of OP exposure, since it may be a useful biomarker of individual susceptibility to OP toxicity (Costa & Furlong 2002).

12.1.1 Metabolic pathway

Commonly used OPs, parathion, chlorpyrifos and diazinon (a major ingredient of sheep dip in the UK during the 1980s and 1990s) are manufactured as organophosphorothioates and are poor inhibitors of acetylcholinesterase (AChE). However, the thioates are converted to highly toxic oxons by liver cytochrome P450 enzymes and the oxon form is a more potent inhibitor of AChE than the parent compound. For example, chlorpyrifos oxon inhibits brain cholinesterase at 1000 times the rate of the parent compound. PON1 hydrolyses the bioactive forms of OPs such as paraoxon, diazoxon and chlorpyrifos oxon, thus limiting toxicity (Brophy et al, 2000, Costa & Furlong 2002, Mackness et al, 2003).

12.1.2 Animal studies

Animal studies have demonstrated the importance of PON1 in modulating OP toxicity. For example studies by Costa & Furlong's research group in the 1990s found that rats or mice, injected with exogenous PON1 (purified from rabbit serum) to increase plasma hydrolytic activity towards OPs, were more resistant than controls to the toxic effects of chlorpyrifos oxon. Later studies utilising PON1 knockout mice, (which have no detectable plasma and liver hydrolytic activity towards various OPs) found knockout mice were killed by dermal exposures to OPs that had no measurable inhibition of brain AChE in normal mice; and injection of pure human PON1 Q192 or PON1R192 to restore plasma PON1, provided protection against the toxicity of diazoxon and chlorpyrifos oxon. These findings have particular clinical relevance to the observation

that newborn humans have very low levels of PON1 in their sera and are particularly sensitive to the toxic effects of OPs (Richter & Furlong 1999).

In recent years increasing attention has been given to biomarkers of susceptibility to OP toxicity and genetic differences in the enzymes involved in the detoxification of OPs can greatly influence their toxicity, hence some individuals may be more susceptible to the toxic effects of OPs than others.

12.1.3 Genotype and human polymorphisms

Polymorphisms of PON1 have been found to modulate the toxicity of OPs. In human populations, two common polymorphisms have been identified in the coding sequence of PON1: L55M and R192Q. The R192Q polymorphism, but not the L55M, affects the catalytic efficiency of hydrolysis of some organophosphate pesticides. For example, the PON1 R192 isoform hydrolyses paraoxon and chlorpyrifos more rapidly than the PON1 Q192 (this is important because up to 50% of the population are homozygous for PON1 Q; Furlong et al 2006); but it metabolises diazoxon more slowly than the Q192 isoform (Cherry, et al, 2011; Davies et al, 1996). As mentioned previously, diazinon was a major ingredient of sheep dip in the UK during the 1980s and 1990s and it is possible that individuals who developed chronic ill health following exposure to OPs carry one or more R alleles.

12.1.4 PON1 activity levels

Costa and Furlong found that possessing efficient PON1 regulatory regions does not alone guarantee a high PON1 activity level, because there are large inter-individual differences in level of PON1 expression. In a given population plasma PON1 activity can vary up to 40 fold and differences up to 13 fold are present within a single PON1 192 genotype (and levels in newborns are 3-4 times lower than in adults).This means that individuals with the same genotype have different levels of protection. Therefore, it is important to measure the level of protein expressed in an individual's plasma in addition to determining genotype. Indeed, studies suggest that plasma levels of PON1 are more important in determining sensitivity to diazoxon exposure than the two PON1 192 alloforms Q or R (Li et al 2000). Thus for some exposures it is only the level of plasma PON1 that is important whereas for others it is both the plasma level of PON1 and the PON1 192 alloform that are important.

Differences in PON1 status (i.e. genotype and PON1 level) may explain why some individuals are more sensitive to certain environmental factors (e.g. organophosphate pesticides), than others and PON1 status may be a better predictor of OP susceptibility than genotype alone. Although animal and in vivo human studies provide evidence that PON1 plays an important role in modulating the toxicity of OPs, only a few studies to date, have looked at the relationship between PON1 status and clinical outcomes in individuals exposed to OPs (Cherry et al, 2002; Cherry et al, 2011; Furlong et al, 2006; Holland et al, 2006; Lee, London, Paulauskis,Myers & Christiani, 2003; Mackness et al 2003; Povey et al, 2005).

12.2 UK studies of sheep dippers

The relationship between exposure to sheep dip, PON1 polymorphisms and chronic ill health has been investigated by Cherry et al (2002 and 2011), Mackness et al (2003) and Povey et al (2005) using a case-referent study design. They investigated the relationship between PON1 genetic polymorphisms and activity levels in farmers reporting chronic ill health attributed to OP exposure in sheep dip (cases) and sheep farmers who carried out similar activities but remained well (controls). They found that the PON1 192 polymorphism was more common in people reporting ill health and that these individuals were more likely to have the R PON1 allele than similarly employed controls who considered themselves to be healthy. Furthermore, cases were more likely than referents to have low serum hydrolytic activity for diazoxon. Indeed, farmers in the lowest quintile of diazoxon hydrolysis were 2.5 times more likely to report ill health (which they attributed to sheep dipping) than farmers in the highest quintile.

12.2.1 The current study

An important objective of the current study was to determine whether background factors such as the capacity to metabolise OPs, render some individuals more vulnerable to the effects of OPs than others. Therefore, each study participant was asked to provide a sample of blood which was shipped to the University of Washington, Seattle for determination of PON1 status by Professor Clement Furlong's laboratory. The methodology for this has been described earlier in Chapter 5. To summarise, participants PON1 status was determined by measuring rates of paraoxon and diazoxon hydrolysis as this correlates with the amount of enzyme activity and protein levels. Their PON1 phenotype (Q/Q; Q/R; R/R) and arylesterase activity levels are summarised

in Table 12.1.

Table 12.1 PON1 status in study participants according to work status.

	Frequency of phenotypes			Arylesterase activity		
	QQ	QR	RR	Mean	SD	Range
Farmers						
Working	26	21	5	150.38	33.67	71-239
Retired	19	26	5	153.85	37.71	88-243
%	44%	46%	10%			
Controls						
Working	16	15	1	143.33	27.10	92-207
Retired	11	11	3	141.09	29.76	91-207
%	47%	46%	7%			

12.2.1.1 Descriptive statistics

The descriptive statistics show that the number of participants in each phenotype group is consistent with what has been reported in other populations. For example, Brophy et al (2001) found that around 9-10% of individuals of Northern European origin were homozygous for the R192 PON1 isoform, 41% were heterozygous for the QR isoform and 49% were homozygous for the QQ isoform.

Mann Whitney U test was carried out on overall ARElase levels between the exposed cohort and the controls. No significant difference was found between farmers and controls in terms of overall ARElase levels (U=2479.5, p=.13), nor when split into working and retired cohorts (U=743, *ns*; U=519, *ns* respectively). This suggests that the farmers are no more susceptible to the neurotoxic effects of OPs than controls.

12.2.1.2 Phenotype and activity levels

Previous research has found the PON1 R192 isoform hydrolyses diazoxon more slowly than the Q192 isoform (Cherry et al, 2011; Davies et al, 1996). However, enzyme activities vary greatly among individuals with the same genotype, so it is important to measure enzyme activity in addition to genotype.

Table12.2 shows the activity levels for the different phenotypes for the participants who took part in the current study.

Table 12.2 PON1 activity and phenotype for all study participants.

	N	Mean	SD
QQ	72	155.78	37.62
QR	73	144.71	29.01
RR	14	131.84	20.56

A one-way ANOVA with Brown-Forsythe correction revealed that there was a significant effect of phenotype on activity levels ($F(2,144.68)=5.42$, $p<.01$) and Games-Howell post hoc tests showed that this was due to the activity in the QQ group being significantly greater than the RR group ($p<.01$). Thus the findings of this study are in line with those previously reported.

12.2.2 How do our findings compare to other studies that have been undertaken around the world? Is there a vulnerable subgroup of farmers with low PON1 activity?

Table 12.3 illustrates that arylesterase levels were slightly higher in this cohort than that reported in other populations (Davies et al 1996; Ekerson, Wyte & La Du 1983; Richter and Furlong 1999; Richter et al. 2009; van Himbergen et al, 2008). Furthermore, no one included in this study had arylesterase levels less than 71 units/ml. This is notably higher than the lowest values reported by several earlier studies which were generally under 50 units/ml (Davies et al 1996; Furlong 2008, unpublished data; Holland et al, 2006). This indicates that there were no poor metabolisers in the exposed cohort examined in the current study (see Figure 12.1). This may have occurred because we inadvertently excluded them from the study.

Participants with a history of acute symptoms following exposure to OPs were excluded from the study in order to restrict the focus to low level exposure. Low level exposure was defined as exposures which do not result in symptoms of acute toxicity. However, it is possible that individuals who experience symptoms of acute toxicity do so because of low PON1 activity levels and not because the level of OP in the environment was unduly high. Four percent of potential study participants were excluded because of a history of acute symptoms following exposure to OPs. In addition, thirteen percent of potential study participants were excluded because they had a history of heart disease or pre-existing neurological problems (e.g. cerebrovascular disease and Parkinson's disease), but low PON1 activity has been associated with these conditions (Costa & Furlong, 2002; van Himbergen et al, 2008; Zintzaras & Hadjigeorgiou, 2004). The

initial reason for excluding these people was to ensure that any cognitive deficits identified in this study were due to OP exposure rather than pre-existing disease, however, it is possible that we inadvertently excluded a sub-group of individuals who are particularly susceptible to the toxic effects of OPs as a result of low PON1 activity.

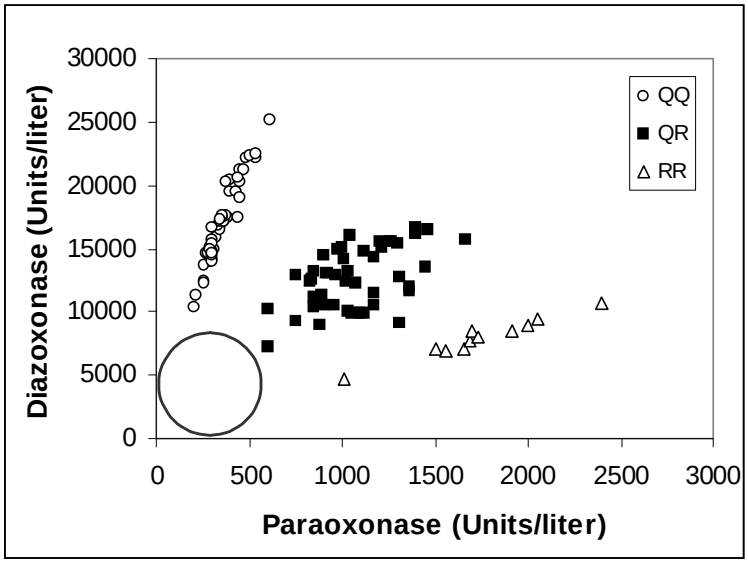


Figure 12.1 PON1 status of sheep farmers with a history of exposure to low levels of OPs in sheep dip.

Table 12.3 Mean ARElase levels reported by earlier studies. Comparison with the findings from the current study.

Study	Population	QQ	QR	RR	Overall mean	Range
Current study 2011	**Farmers**	**165.33 (38.82) (n=45)**	**143.59 (30.39) (n=47)**	**132.37 (20.68) (n=10)**	**152.08 (35.58) (n=102)**	**71-243**
	Controls From UK	**139.87 (29.92) (n=27)**	**146.74 (26.81) (n=26)**	**130.50 (23.34) (n=4)**	**142.34 (28.06) (n=57)**	**91-207**
Furlong 2008*	Navy Seals	136.1 (34.09) (n=370)	132.9 (30.16) (n=352)	116 (31.10) (n=106)	MD	<50-275
Davies et al 1996	Hispanics	138 (37) (n=78)	131 (28) (n=41)	145 (32) (n=18)	136 (32) (n=92)	57-235
Holland et al 2006	Latino mothers from USA	151.9 (n=39)	144.3 (n=61)	152.2 (n=30)	149.2 (n=130)	19.8-281.4
van Himbergen et al 2008	Postmenopausal women from the Netherlands	84 (24) (n=785)	89 (25) (n=589)	94 (25) (n=140)	87 (25) (n=1527)	MD
Ekerson et al 1983	Controls from USA	116 (33) (n=159)	115 (30) (n=144)	121 (29) (n=45)	MD	~25 - ~245
Wills et al 2010	ALS patients	MD (n=68)	MD (n=55)	MD (n=17)	150.3 (38.9) (n=140)	MD
	Controls From USA	MD (n=82)	MD (n=61)	MD (n=10)	142.1 (35.5) (n=293)	MD

*unpublished data from Professor Furlong's laboratory. ALS = Amyotrophic lateral sclerosis; MD some data is missing as not all studies report all the data listed in the table.

12.3 Is there a relationship between dippers flu and PON1?

12.3.1 Activity

A number of participants (33.8% of the working cohort and 50.9% of the retired group) reported that throughout their working life they suffered repeated episodes of 'dippers flu' which may reflect undiagnosed, untreated acute toxicity. To test the hypothesis that study participants who report dippers flu may represent a vulnerable subgroup of farmers with low PON1 activity, a comparison of ARElase levels amongst participants who did an did not report a history of dippers flu was undertaken.

ARElase levels were similar for participants who reported dippers flu, versus those who did not (overall - U=2162, *ns*; working - U=499.5, *ns*; retired - U=541, *ns*).

Participants who reported a history of dippers flu were also asked to estimate how frequently they suffered from it during their working lives and the options they could choose from were: every time they dipped sheep, most times, sometimes, not often, or once or twice. A Spearman correlation revealed no significant relationship between frequency of dippers flu and ARElase levels (r_S=.05, *ns)*.

12.3.2 Phenotype

To investigate the potential role that PON1 phenotype may play in the incidence of dipper's flu, the number of participants who reported such symptoms was investigated for each of the three subgroups (QQ, QR, RR). While 35.5% of participants with the QQ phenotype reported a history of dipper's flu, this was true for 44.4% of QR participants and 60% of the RR group.

As previous research has suggested that participants with the RR allele may be worse at metabolising OPs, odds ratios were calculated for the incidence of dipper's flu for participants who phenotype RR compared to QQ. It was found that participants with the RR allele were 2.7 times more likely to report having dipper's flu than those with QQ isoforms.

12.4 Relationship of PON1 status and cognition in the exposed participants

12.4.1 Activity

To investigate the relationship between PON1 activity and cognition, Spearman correlations were run between ARElase levels and cognitive domain z-scores (see Table 12.4). Only two nominally significant correlations were found. The first was between ARElase levels and auditory memory, although it was in the opposite direction to what would be expected in that participants with higher ARElase levels obtained lower scores on tests of auditory memory. The other significant relationship was in the dippers flu group. Participants with higher ARElase levels performed better on a test of fine motor skills than those with lower ARElase levels. However, while these findings were nominally significant, only the latter survived Larzelere and Mulaik corrections.

Table 12.4 Relationship between PON1 activity and cognitive domain z-scores.

	All exposed	Dippers Flu	No Dippers Flu
Working Memory	-0.01	-0.05	0.05
Visual Memory	-0.05	-0.02	-0.08
Auditory Memory	-0.21*	-0.08	-0.26
Response Speed	0.11	0.20	0.05
Verbal Ability	-0.06	-0.15	0.01
Mental Flexibility Inhibition	0.08	0.10	0.04
Strategy Making	0.06	0.05	0.05
Verbal Visual Reasoning	-0.11	-0.20	-0.07
Visio-Spatial Ability	-0.03	-0.11	0.00
Fine Motor Control	0.16	0.52***	0.06

Asterisks denote 2-tailed significance: *** - p<.001; * - p<.05

Further analyses were undertaken in which the exposed cohort were split into upper and lower levels of ARElase based on a mean and median split. A series of Mann Whitney U tests found no significant differences between participants with high and low PON1 levels in any of the cognitive domains in terms of mean, median or quintile split. The same tests were carried out for exposed participants both with and without dippers flu. Only one significant result was found: for participants with dippers flu, participants with high ARElase levels based on a median split had significantly better fine motor skills (mean z-score = .42, SD = .50) than those with lower PON1 levels (mean z-score = -.14, SD = .79; U=331.5, p=.005).

12.4.2 Phenotype

To investigate the difference in cognitive ability for each of the PON1 phenotypes, a selection of one way ANOVAs for these three subgroups (QQ, QR, RR) were carried out on each of the 10 cognitive domain z-scores. None of the results were significant (largest F=1.36). However, given that there were only 10 participants in the RR group it is not surprising that no effects were found. Therefore, the possible effect of phenotype on cognitive performance was investigated in a different manner.

The performance of participants on each of the 10 cognitive domains were split into quintiles, and the proportion of participants falling into the lower quintile were looked at in relation to their PON1 phenotype. This can be seen in Figure 12.2.

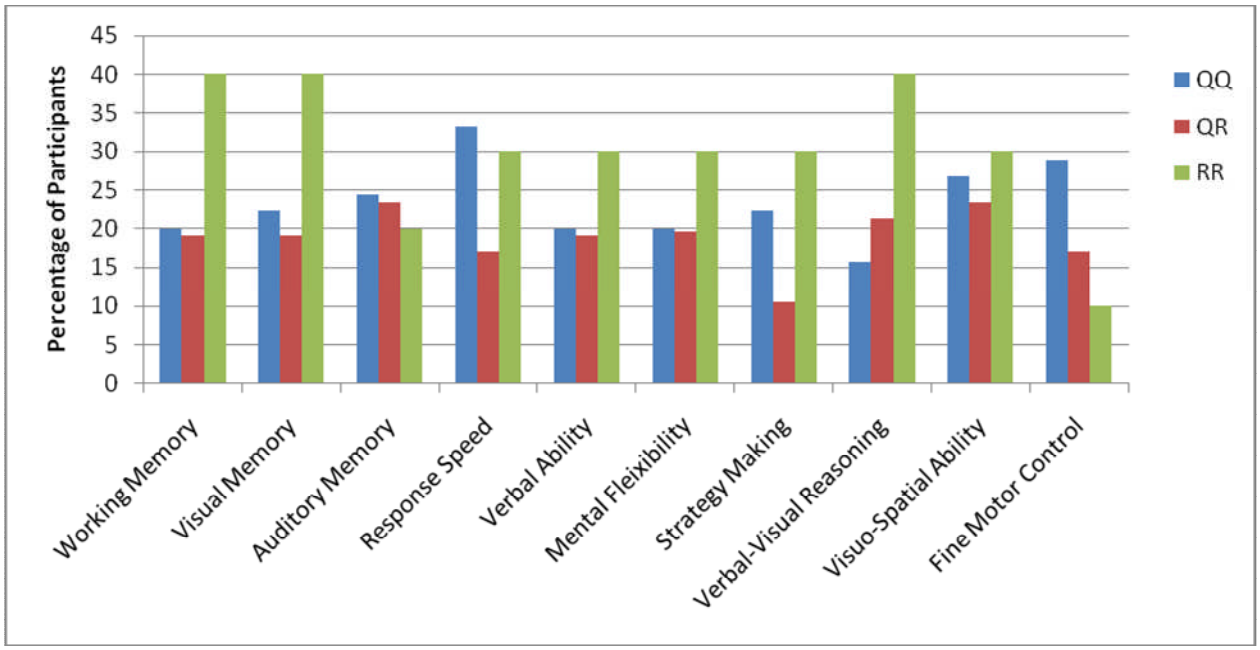


Figure 12.2 Proportion of participants within each phenotype falling into the bottom quintile of performance for each of the cognitive domains.

As before, the role of phenotype was investigated by calculating odds ratios were for each of the 10 cognitive domains for participants who phenotype RR compared to QQ (these can be seen in Table 12.5). An odds ratio greater than 1 indicates that the participant is more likely to fall into the lowest quartile when they are in the RR group. In contrast, an odds ratio less than 1 indicates being in the lowest quartile of performance is more prevalent in the QQ group.

Results showed that in seven out of ten of the cognitive domains, participants with the RR isoform were more likely to fall into the bottom quintile than the QQ participants. In fact, the odds of being in the bottom quintile in the RR group were more than double that for the QQ group on measures of Working Memory, Visual Memory and Verbal-Visual Reasoning. However, the opposite was true for Fine Motor Control.

Table 12.5 ODDs ratios for each of the 10 cognitive domains investigating RR versus QQ.

Cognitive Domain	ODDS Ratio
Working Memory	2.67
Visual Memory	2.34
Auditory Memory	0.77
Response Speed	0.86
Verbal Ability	1.71
Mental Flexibility	1.71
Strategy Making	1.50
Verbal-Visual Reasoning	3.61
Visuo-Spatial Ability	1.18
Fine Motor Control	0.27

12.5 Relationship of PON1 status and mood in the exposed participants

12.5.1 Activity

To investigate the relationship between PON1 activity and mood, Spearman correlations were run between ARElase levels and HADS, BDI and BAI scores. No significant correlations were found.

As before, further analyses were undertaken in which the exposed cohort were split into upper and lower levels of ARElase based on a mean, median or quintile split. A series of Mann Whitney U tests found no significant differences between participants with high and low PON1 levels for any of the measures of mood.

12.5.2 Phenotype

To investigate the potential influence of PON1 phenotypes on mood, a selection of one way ANOVAs for these three subgroups (QQ, QR, RR) were carried out on each of the anxiety and depression measures. Again, none of the results were significant.

As before, the role of phenotype was investigated by looking at the number of participants who score above clinical cut-offs for depression and anxiety for each

isoform group, and calculating odds ratios (which can be seen in Table 12.6). An odds ratio greater than 1 indicates that the participant is more likely to score above clinical cut-offs on mood measures when they are in the RR group. In contrast, an odds ratio less than 1 indicates the opposite.

Results showed that participants with the QQ isoform were more likely to report problems with mood than the RR participants. This is the opposite of was expected.

Table 12.6 ODDs ratios for mood in RR versus QQ phenotypes.

Measure	Odds Ratio
BDI	.36
BAI	.40
HADSA	.68
HADSD	.92

12.6 Relationship of PON1 status and physical symptoms in the exposed participants

12.6.1 Activity

To investigate the relationship between PON1 activity and physical symptoms, Spearman correlations were run between ARElase levels and overall health rating, measure of pain, impact on social life, and reported severity of the 10 symptoms of interest identified in the previous chapter. No significant correlations were found.

As before, further analyses were undertaken in which the exposed cohort were split into upper and lower levels of ARElase based on a mean, median or quintile split. A series of Mann Whitney U tests found no significant differences between participants with high and low PON1 levels for any of the physical measures.

12.6.2 Phenotype

To investigate the potential influence of PON1 phenotypes on physical symptoms, a selection of one way ANOVAs for these three subgroups (QQ, QR, RR) were carried out on each of the physical symptom measures outline in 11.7.1. None of the results were significant.

As before, the role of phenotype was investigated by looking at the frequency of symptomatic participants for each of the 10 physical measures for each isoform group.

This can be seen in Figure 12.3.

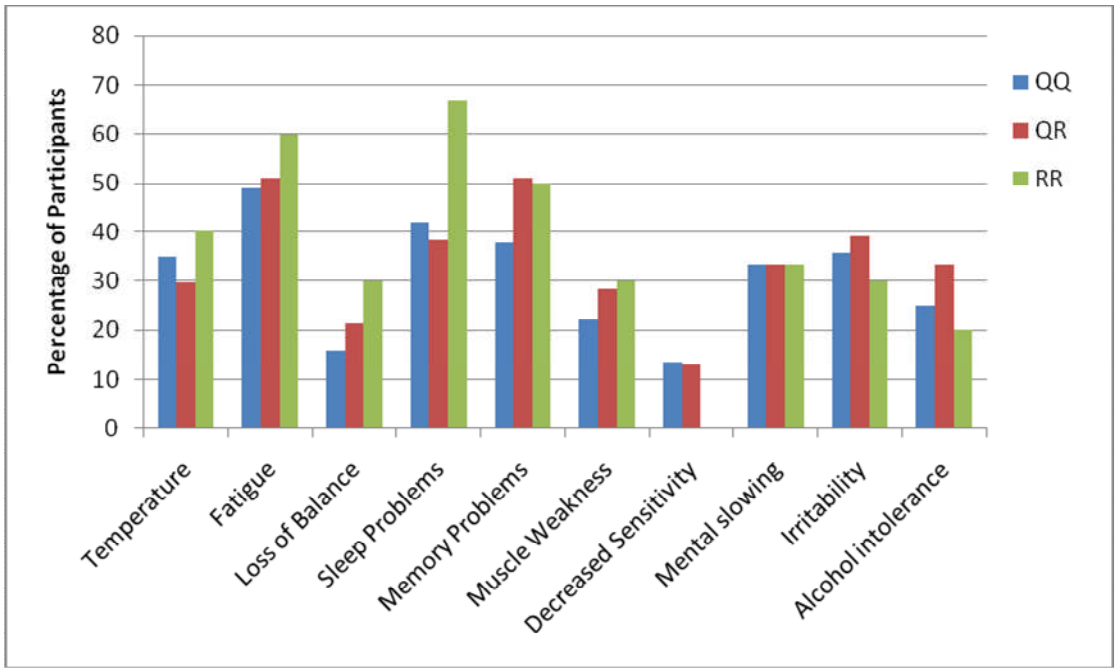


Figure 12.3 Proportion of symptomatic participants within each phenotype.

Again, odds ratios were then calculated for each of the 10 domains for participants with phenotype RR compared to QQ (these can be seen in Table 12.7). An odds ratio greater than 1 indicates that the participant is more likely to be symptomatic when they are in the RR group. In contrast, an odds ratio less than 1 indicates the opposite.

Results showed that for seven of the ten physical symptoms participants with the RR isoform were more likely to report being symptomatic than the QQ participants. In fact, the odds of being in the bottom quintile in the RR group were more than double that for the QQ group for Loss of Balance and Sleep Problems.

Table 12.7 ODDs ratios for each of the 10 key physical symptoms investigating RR versus QQ.

Symptom	Odds Ratio
Temperature	1.24
Fatigue	1.57
Loss of Balance	2.27
Sleep Problems	2.78
Memory Problems	1.65
Muscle Weakness	1.50
Decreased Sensitivity	n/a
Mental slowing	1.00
Irritability	0.78
Alcohol intolerance	0.75

12.7 Summary

Paraoxonase (PON1) is an enzyme which metabolises oxidized lipids and organophosphate pesticides such as chlorpyrifos, parathion and diazinon. Genetic polymorphisms of PON1 have been identified in the coding sequence of PON1 (R192Q) which differ greatly in their activity towards different organophosphate pesticides and modulate the toxicity of OPs. The R 192 isoform hydrolyses paraoxon and chlorpyrifos more rapidly than the Q192; but metabolises diazoxon (the toxic metabolite of diazinon, an OP used in sheep dip in the UK) more slowly than the Q192 isoform. Cherry et al (2002 and 2011) found that the PON1 R192 polymorphism was more common in sheep farmers reporting ill health than those who are healthy; and ill farmers were more likely than healthy farmers to have low serum hydrolytic activity for diazoxon.

An important objective of the current study was to determine whether differences in the capacity to metabolise OPs render some individuals more vulnerable to the effects of OPs than others. Results revealed that the number of participants who fell into each phenotype group is consistent with what has been reported in other populations, but mean ARElase levels were higher as were the lowest values noted. This indicates that there were no poor metabolisers in the exposed cohort, something which may have occurred because our exclusion criteria included diseases associated with low PON1 activity. No relationships were found between PON1 activity levels neurobehavioural

and/or health outcomes, possibly because our distribution/range of AREase values was restricted. However, analysis by phenotype revealed lower PON1 activity in the RR group and individuals with the RR isoform were more likely to fall into the lowest quintile of performance on cognitive tests, particularly working and visual memory and reasoning tasks; and were more likely to complain of physical symptoms associated with exposure to OPs.

Chapter 13 : Meta-analysis Revisited

13.1 Overview

In Chapters 3 and 4 a systematic review of the literature was undertaken along with quantitative evaluation of study findings using meta-analysis. Meta-analysis is a useful method of quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. In this section the meta-analysis is repeated, incorporating the findings from the current study. Figure 13.1 is a forest plot depicting effect sizes from the initial 12 studies described in Chapters 3 and 4 plus the findings from the current study.

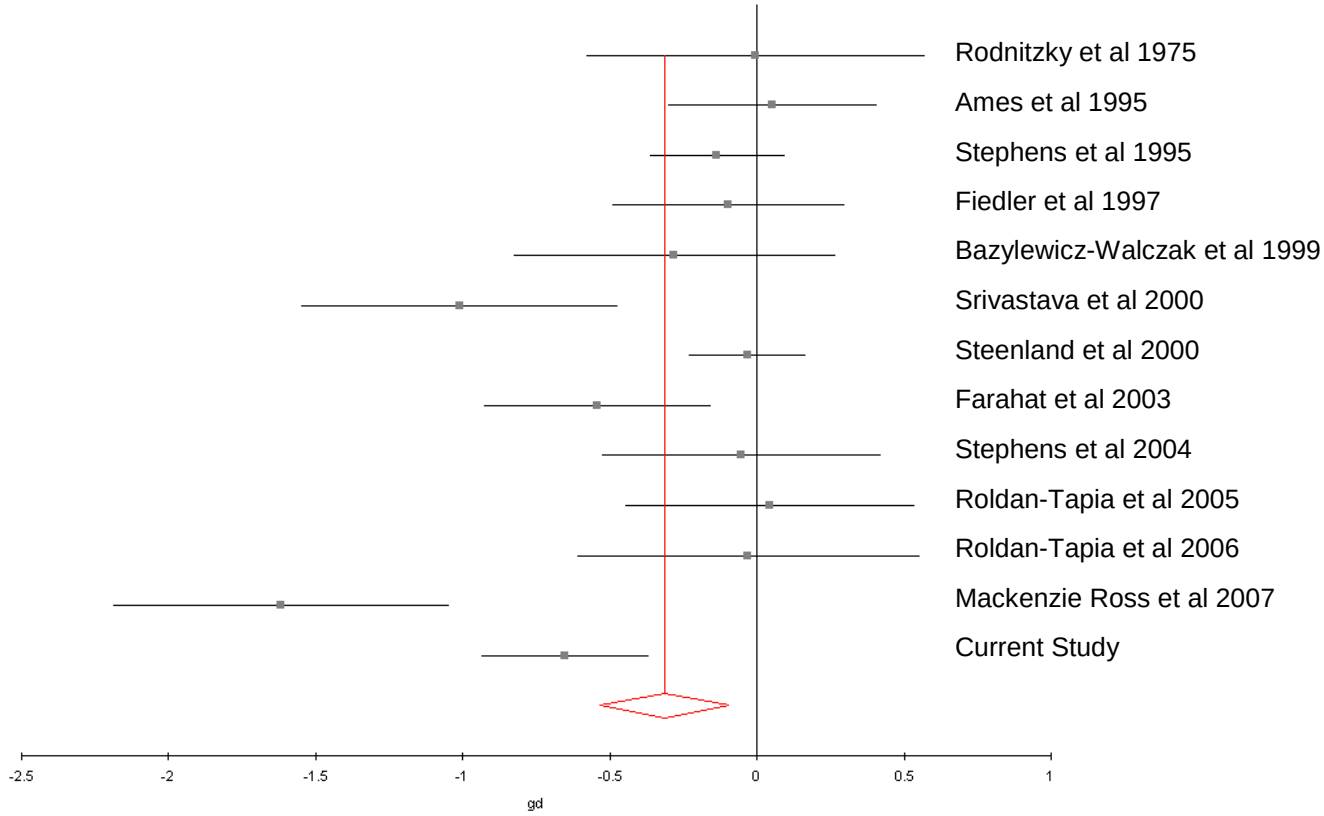


Figure 13.1 Forest plot depicting effect sizes for each of the studies in date order and 95% confidence intervals.

The overall effect size for the current study (based on Glass's delta and the population mean) is moderate *ES* = - 0.652. Most of the effect sizes illustrated in Figure 13.1 cluster around-.03 (overall ES -0.3148, $p<0.0053$) and the findings from the current study are in keeping with those from earlier studies. Srivastava et al (2000) and Mackenzie Ross et al (2007) studies remain the ones which produce the largest effect sizes. Since the earlier study by Mackenzie Ross et al may be biasing the overall

findings, all subsequent analyses and results illustrate the effect of including and excluding that study on the overall findings.

Table 13.1 Meta-analysis using a random effects model illustrating the effect of including the findings from the current study and excluding the earlier study by Mackenzie Ross et al (2007).

	Glass Delta Mean Current findings included. *Mackenzie Ross et al 2007 excluded*	Glass Delta Mean All 13 studies included
Overall ES	-0.2251	-0.3148
95% CI lower	-0.402	-0.5361
95% CI upper	-0.0482	-0.0934
z	2.4939*	2.7867**
t^2	0.0541	0.1168

-Asterisks denote significant effects: * p<.05; ** p<.01

Excluding the study by Mackenzie Ross et al (2007) does not render the overall findings non significant, but does result in a large reduction in the heterogeneity rating. Removal of the study alters the overall balance and comparability of remaining studies which appear more homogeneous once it has been excluded; but the overall effect size produced by the meta-analysis remains significant. The convention with regard to interpreting effect sizes is that d=0.2 to 0.5 is 'small'; 0.5-0.8 is medium and >0.8 is large; hence the overall effect size found in the current analyses of between -0.2251 and -0.3148 (depending upon whether the study by Mackenzie Ross et al 2007 is included or not) can be classified as small. The fail safe N, which estimates the number of studies with a zero effect needed to make the results of the current meta-analysis non significant, is 84.

13.2 Effect of cognitive task

Thus far the meta-analysis has incorporated data from all of the psychometric tests administered in a given study (i.e. multiple effect sizes were calculated) and then a single mean effect size within each study was computed before undertaking the meta-analysis. To determine whether task parameters might influence effect sizes the meta-

analysis was repeated but this time cognitive tests were grouped into cognitive domains and a single effect size was calculated for each domain by averaging the effect sizes across all measures within that domain. Table 13.2 summarises the results of meta-analysis by cognitive domain. For each domain, the first row illustrates the effect sizes produced by all studies whilst the second row illustrates the findings when the study by Mackenzie Ross et al (2007) which produces the largest effect sizes, is removed.

Table 13.2 Meta- analyses by cognitive domain (italic illustrate the findings when the study by Mackenzie Ross et al (2007) is removed).

Cognitive Domain	**No studies**	**Overall ES**	**Lower CI**	**Upper CI**	**z**	**t^2**
Working Memory	12	-0.338	-0.595	-0.080	2.568*	0.156
	11	*-0.266*	*-0.511*	*-0.022*	*2.134**	*0.123*
Visual Memory	10	-.0297	-0.532	-0.062	2.475*	0.096
	9	*-0.217*	*-0.421*	*0.014*	*2.096**	*0.054*
Verbal Memory	9	-0.152	-0.486	0.182	0.893	0.214
	8	*-0.007*	*-0.218*	*0.233*	*0.062*	*0.065*
Attention	9	-0.263	-0.511	-0.014	2.078*	0.099
	8	*-0.142*	*-0.325*	*-0.041*	*1.524*	*0.031*
Speed	13	-0.531	-0.899	-0.163	2.825**	0.407
	12	*-0.320*	*-0.541*	*-0.100*	*2.848***	*0.106*
Executive function	10	-0.399	-0.796	-0.002	1.969*	0.361
	9	*-0.201*	*-0.504*	*0.101*	*1.304*	*0.171*
Visuo-spatial	5	-0.370	-0.616	-0.123	2.938**	0.029
	4	*-0.295*	*-0.531*	*-0.060*	*2.455**	*0.015*
Language	7	-0.267	-0.548	0.014	1.864	0.093
	6	*-0.105*	*-0.249*	*0.039*	*1.433*	*0.002*
FMC	4	-0.462	-1.075	0.150	1.480	0.354
Mood	5	-0.517	-1.044	0.012	1.920	0.310

-Asterisks denote significant effects: * p<.05, ** p<.01 and ***p<.001

As before, the neuropsychological tests which produced the largest effect sizes included tests of working memory (digit span), psychomotor speed and visuo-spatial ability. However, the initial analyses found tests of fine motor control produced significant effect sizes but the current analysis did not and instead found visual memory tests and mood measures produced significant effect sizes. Figure 13.2 shows stem and leaf displays of effect size data for the cognitive domains which produced the largest effect sizes during the current analysis. Table 13.3 illustrates similarities and differences in findings between the initial and current meta-analyses.

Psychomotor speed (12 studies)

Stem	Leaf
1	
0	0,0,0,1
-0	0,1,1, 2,5,6,7,8,8
-1	

Working memory (11 studies)

Stem	Leaf
1	
0	0,0,1,1,1
-0	1,2,4,6,7
-1	3

Visual memory (9 studies)

Stem	Leaf
1	
0	0,0
-0	0,1,1,1,4,5,7
-1	

Visuo-spatial ability (4 studies)

Stem	Leaf
1	
0	
-0	1,4,5,5
-1	

Mood (5 studies)

Stem	Leaf
1	
0	1,3
-0	5,9
-1	2

Figure 13.2 Stem and leaf displays of the effect size data for the cognitive domains which produced the largest effect sizes (Mackenzie Ross et al study removed).

Table 13.3 Neuropsychological tests producing the largest effect sizes.

Initial meta-analysis (Chapter 3)	**Revised meta-analysis**
Working memory	Working memory
Speed	Speed
Visuo-spatial ability	Visuo-spatial ability
Fine motor control	Verbal memory
	Mood

13.3 Summary

In Chapters 3 and 4 the findings from a systematic review of the literature investigating the functional consequences of long term low level exposure to OPs were reported. The majority of well designed studies found a significant association between long term, low level exposure to OPs and impaired neurobehavioural function, which was small in magnitude and concerned primarily with neurobehavioural functions such as working memory, psychomotor speed, fine motor control and visuo-spatial ability. In this chapter meta-analyses were repeated, incorporating the findings from the current study, to determine whether the findings are consistent with earlier work.

The overall effect size for the current study was found to be moderate in size and slightly higher than that found in the majority of previous studies, but lower than studies by Srivastava et al (2000) and Mackenzie Ross et al (2007) which produced moderate to large effect sizes.

When the findings from the current study were included into a meta-analysis which assimilated the results from 13 studies and over 1500 subjects, a significant association was found between exposure to low levels of OPs and decrements in cognitive function, which is small in magnitude and consistent with the findings reported in Chapters three and four.

Earlier research suggests some cognitive functions are affected to a greater degree than others by exposure to OPs and tests of psychomotor speed, reaction time, fine motor

control, attention and memory are particularly sensitive to OP exposure; and non verbal abilities tend to be affected to a greater degree than verbal abilities which do not appear sensitive to neurotoxic effects (Anger, Otto & Letz, 1996; Anger et al, 1997; Anger et al, 2000; Hartman 1995; Lucchini et al 2005). The finding of the current meta-analysis were in broad agreement with previous research in terms of the neurobehavioural domains affected which included slowing of reaction times and reduced performance on tests of working memory (digit span) and visuo-spatial ability. Consistency of findings across many studies strongly suggests the association between long term, low level exposure to OPs and impaired neurobehavioural function is real and unlikely to be due to alternative explanations. For example, impairment due to psychosomatic disorder, malingering or stress would be more likely to produce a pattern of global deficit or variable, inconsistent symptom profiles. It seems reasonable to conclude that low-level exposure to organophosphates has subtle and specific effects on the central nervous system, resulting in neurobehavioural problems which may not be apparent to health care professionals unless patients undergo formal evaluation utilizing sensitive neuropsychological tests.

Chapter 14 : Discussion

14.1 Overview

This chapter will provide an overview of the findings of the empirical study described in this thesis. Results will be discussed with respect to the study objectives described in Section 5.1.1 and existing literature in this area. Potential limitations of the study design and sources of bias will then be considered following which the implications of the study findings and directions for future research will be explored.

The primary objective of this thesis was to determine whether low-level exposure to OPs (insufficient to cause acute intoxication) is associated with disabling neuropsychological and psychiatric disease. The initial step taken to address this question involved carrying out a systematic review of the existing literature concerning the functional consequences of long-term low-level exposure to OPs, described in Chapters 3 and 4. Previous research has produced inconsistent findings, possibly because the large body of literature that exists concerning the neurotoxicity of OPs incorporates a range of different methodologies, populations examined and outcome measures. While some studies find evidence of ill health and cognitive impairment following low level organophosphate exposure (Amr et al, 1997; Bazylewicz-Walczak et al, 1999; Farahat et al, 2003; Mackenzie Ross et al, 2007; Roldan-Tapia et al 2005 and 2006; Steenland et al, 2000; Srivastava et al, 2000; Stephens & Sreenivasan, 2004; Stephens et al, 1995), others do not (Ames et al, 1995; Fiedler et al, 1997; Jamal et al, 2001; London et al, 1997; Rodnitzky et al, 1975).

To date, the only published reviews investigating the potential link between low-level OP exposure and neuropsychological deficits have been qualitative (Alavanja et al, 2004; Arcury & Quandt, 1998; Brown & Brix, 1998; Colosio et al, 2003; COT report, 1999: Davies, 1990; De Silva et al, 2006; ECETOC Report 1998; Jamal et al, 2002; Kamel & Hoppin, 2004; OCFP Report, 2004; Ray, 1998; Royal Colleges' Report, 1998; Soltaninejad & Abdollahi 2009). As such, this thesis sought to extend these findings by including a quantitative evaluation of previous study findings using meta-analysis. Meta-analysis is a useful method of quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. It does this by representing each study's findings in the form of effect sizes which are a

statistical standardisation of study findings based on standard deviation units. Combining information across studies in this way increases statistical power to detect small effects that may be missed by individual studies which are too small to yield a valid conclusion (Zhou et al, 2002).

Data from more than 1,400 participants was aggregated in order to produce a more reliable estimate of the association between exposure to OPs and neuropsychological impairment. The analyses described in Chapter 4 showed that the majority of well designed studies find a significant association between long-term, low-level exposure to OPs and impaired cognitive function, which is consistent, small to moderate in magnitude and concerned primarily with neurobehavioural functions such as working memory, psychomotor speed, fine motor control and visuo-spatial ability. However, a number of unresolved issues remain in the literature concerning the precise nature of the relationship between exposure to OPs and neurobehavioural function and whether the strength of the association has been under- or over-estimated.

In order to address methodological weaknesses of earlier work, an empirical study was designed and undertaken as part of this thesis. Findings from this four year empirical study are described in Chapters 5-13. The study involved a comparison of neuropsychological performance in 127 UK sheep farmers with a history of low-level exposure to organophosphate pesticides and 78 non-exposed controls (matched for age, gender, years in education and intellectual ability). Information was also obtained about physical and mental health. Study participants who had retired on ill health grounds were included to take account of the 'healthy worker effect', something previous studies had failed to do. Participants with a history of acute poisoning and those with a psychiatric or medical history that might otherwise account for ill health were excluded; exposure history was examined in detail and objective, reliable, valid, neuropsychological tests were used which are known to be sensitive to neurotoxic effects. Finally genetic factors that may render some individuals more vulnerable to the effects of OPs than others were explored. The primary aim of this empirical study was to establish whether farm workers with a history of low-level exposure to OPs show evidence of physical disease, cognitive impairment and / or mood disorder; and to determine the nature and severity of any deficits or symptoms identified.

14.2 Findings in relation to primary objectives

A range of emotional, physical and cognitive problems were identified in sheep farmers with a history of low level exposure to OPs and are described below:

14.2.1 Cognitive function

In terms of cognitive function, general intellectual ability, reasoning, visuo-spatial and verbal ability were relatively well preserved, but sheep farmers obtained lower scores on tests of response speed, working, verbal and visual memory, mental flexibility and fine motor control, than non-exposed controls. These differences remained after controlling for Type I errors. Few differences were found between working and retired farmers in terms of the cognitive deficits identified.

14.2.1.1 Possible confounding factors

While the results described above suggest that long-term, low-level exposure to OPs causes a significant pattern of cognitive deficit, it is possible that the findings were caused by extraneous variables. Previous research has shown that depression and anxiety can significantly alter cognitive performance and so it is standard practice to take account of participants' mood scores when analysing their performance on neuropsychological tests. To examine whether the patterns of deficit observed in the initial analysis could have been driven by the potentially confounding effects of anxiety and depression on cognitive performance, the data was re-analysed after controlling the effects of mood statistically or removing all farmers who scored in the clinical range for anxiety and/or depression on a self report measure (HAD). The same areas of deficit remained after the effects of anxiety and depression were removed.

A second possibility is that the pattern of deficit observed in sheep farmers was driven by accidental inclusion of study participants with a history of undiagnosed acute exposure. To determine whether this was the case the data was re-analysed after removing all farmers who had reported a history of 'dippers flu'. The same areas of deficit remained even after removal of these subjects.

A third possibility is that the pattern of deficits identified in sheep farmers was due to the use of an inappropriate control group (i.e. rural police workers) who differ from farmers in some important way other than exposure history, resulting in exaggerated group differences. To determine whether this was the case, the performance of sheep

farmers on neuropsychological tests was compared to test norms derived from a cross section of healthy adults in the general population. The same pattern of deficit was evident whether sheep farmers were compared to rural police workers or a cross section of healthy adults from the general population.

It therefore seems reasonable to conclude that the lower performance observed in exposed farmers relative to unexposed controls, on neuropsychological tests, is unlikely to have occurred by chance or to be due to the confounding effects of mood disorder, undiagnosed acute exposure or selection of an inappropriate control group.

14.2.2 Mood disorder

In terms of emotional well-being, several measures of mood were included in this study. Rates of depression and anxiety were much higher amongst farmers than controls, especially amongst retired farmers. Rates differ according to the method used to evaluate mood in that a smaller percentage of farmers were found to meet DSM-IV criteria for a diagnosis of anxiety or depression than the numbers who complain of significant levels of distress on self-report screening tools.

An important issue is the extent to which the mood disorder observed in farmers is due to exposure to OPs as opposed to lifestyle factors. No significant correlations were observed between indices of exposure to OPs and mood raising the possibility that OP exposure is not an important aetiological factor in the development of anxiety and depression. Retirement per se is unlikely to be solely responsible for the mood disorder observed in retired farmers as controls who had also retired on ill health grounds had lower rates of mood disorder than farmers. Stressful life events, although associated with increased severity of mood disorder, are unlikely to be solely responsible for elevated rates of anxiety and depression in farmers as the rate of recent stressful life events is equivalent in the exposed farmers and unexposed controls. Rather, elevated rates of anxiety and depression reported by sheep farmers appear to be related to deteriorating physical health and well being.

14.2.3 Physical health

In terms of physical health, farmers who took part in this study reported significantly worse general health than controls and an increased number of moderate to severe symptoms. The ten physical symptoms which readily distinguished the exposed and

control groups were fatigue, mental slowing, memory problems, irritability, sleep problems, reduced sensitivity (parasthesia), muscle weakness, loss of balance, temperature dysregulation, and alcohol intolerance. These are consistent with those reported in earlier studies (Ahmed & Davies 1997; Beach et al, 1996; Davies et al, 1999; Dunn, 2002; Fletcher et al, 2005; Jamal et al, 2002; Mackenzie Ross et al, 2007; Pilkington et al, 2001; Solomon et al, 2007; Tahmazet al, 2003). However, the frequency with which they suffered from these symptoms did not correlate significantly with indices of exposure. Nevertheless, the odds of a participant being symptomatic were higher for those in the highest exposure quintile than those who were not and this suggests OP exposure may indeed have a role to play in physical health, but the relationship may not be linear.

14.2.4 Dose effect relationship

As stated above, the primary aim of the study was to establish whether farm workers with a history of low-level exposure to OPs (insufficient to cause acute intoxication) show evidence of physical disease, cognitive impairment and / or mood disorder. Although differences were noted between exposed and unexposed study participants these could have been caused by confounding factors which were not measured or controlled for in the study. Therefore, the relationship between indices of exposure and measures of cognitive function, mood and physical health was explored.

Unfortunately, there is no biomarker of chronic, long term exposure to organophosphate pesticides so it was not possible to quantify levels of exposure or analyse precise dose/response relationships by objective means. Instead, exposure had to be estimated via self-report. Measuring exposure in this way may be problematic as self report may be distorted by inaccuracies of memory and response bias (e.g. a tendency to over or underestimate). Given farmers in this study were being asked to provide details of work history extending back over the course of their lifetime and given farmers in this study showed evidence of memory impairment, the accuracy of the exposure information they provided is open to question, thus reducing the chance of finding significant or reliable associations.

Farmers were asked to complete a questionnaire regarding their exposure history. This revealed that study participants had been exposed to OPs through sheep dipping for an average of 24 years and farmers typically dipped once or twice a year for one to two

days. However, there was a degree of variability amongst individuals in terms of their exposure history with some participants deviating from the normal pattern on one or more variables such as duration, frequency or intensity of exposure. Exposure metrics were constructed in an attempt to consider these different aspects of exposure history simultaneously. However, the exposure indexes also produced variable results raising questions about their validity.

Statistical analyses were undertaken to determine whether a linear relationship exists between indices of exposure and cognitive function. A number of significant, negative correlations were found between duration of exposure (but not the exposure metrics) and visual memory, response speed, verbal ability, mental flexibility, strategy making and verbal-visual reasoning. However these were lost following statistical correction for Type I error. This can be interpreted in two ways (1) the cognitive deficits identified in farmers were not caused by exposure to OPs but by some other confounding factor which was not measured in this study (2) the cognitive deficits identified in study participants were caused by exposure to OPs, but may have been lost for one (or more) of the following reasons:

- The association was small and eradicated following the application of overly conservative statistical methods.
- The study did not include a sufficient number of participants to reliably detect small effect sizes.
- Accurate estimation of exposure was impossible and critical exposure data was not captured, rendering exposure measures invalid.
- The relationship between exposure to OPs and neurobehavioural functioning is not linear.

14.2.4.1 Statistical correction

As mentioned previously, a debate exists in the literature concerning the need to make p-value adjustments when multiple outcome measures have been used, as this increases the risk of Type II error, particularly when subtle or rare effects are being investigated (Feise, 2002; Moran, 2003). Some researchers recommend adjusting the p-values when multiple measures have been used to reduce the risk of finding spurious, false positive

results which have occurred by chance (Bland & Altman, 1995; Ludbrook, 1998; Rice, 1989). Other authors argue that adjustments such as Bonferroni are overly conservative and in reducing the chance of making a Type I error, the risk of Type II error is increased (Feise, 2002; Moran, 2003). Moran (2003) points out that the probability of finding several consistent significant results in a study and for all of them to be due to chance is low, and although spurious results are likely when multiple measures are used, Moran asserts that these should not concern researchers as they are unlikely to be replicated in future studies. He goes on to argue that the application of statistical correction when multiple outcome measures are used is tantamount to punishing a researcher for undertaking detailed work as it lowers the probability of finding significant results; and furthermore, there is no consensus on when to apply statistical correction. For example there is debate regarding whether corrections should be restricted to specific sub-sections of results, whether they should be applied to all of the results from a study in one go, whether they should be applied to all papers published in a journal, or to all of the research undertaken on a given topic (Feise, 2002; Moran, 2003). Clearly the latter would make it impossible to ever reject the null hypothesis for a topic under investigation.

Feise (2002) and Moran (2003) suggest study findings should be interpreted within the context of study design, methodology, sample size and whether the results are replicated by others, rather than relying on overly conservative statistical methods. Austin Bradford-Hill, a medical statistician, devised criteria to assist researchers in determining whether significant results are due to real biological effects rather than random chance (Bradford-Hill, 1965). The main principles set forward by Bradford-Hill, which he suggests researchers should consider before concluding there is adequate evidence of causation, are:

(1) The strength of the association observed between two variables - the stronger the association between two variables the less likely it is that the relationship is due to chance or extraneous variables. However, a small association does not mean there is not a causal relationship.

(2) Consistency of findings – previous research should report similar findings.

(3) Biological gradient – there should be some sort of dose response relationship, in that greater exposure should be associated with greater risk.

(4) Temporal sequence – exposure should precede the outcome for it to have a causal relationship with it.

(5) Biological or theoretical plausibility – there should be a plausible mechanism between cause and effect, consistent with current understanding of underlying processes.

(6) Coherence with established knowledge – any association between variables should be compatible with existing theories, hypotheses and research evidence.

(7) Specificity of the association – the cause should be tightly linked to outcome and alternative explanations should be ruled out.

Although the correlations reported in this study were weak, they were in the expected direction and exhibited a dose response relationship in that greater exposure was associated with lower performance. The findings were consistent with study hypotheses and group analyses; and the results were consistent with previous research involving UK sheep farmers (Mackenzie Ross et al, 2007; Solomon et al, 2007; Stephens, 1995). The possibility that low level exposure to OPs may cause neurobehavioural problems is also biologically plausible (see Chapter 2) and of particular interest here 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.

There are a large percentage of cholinergic nerves in the hippocampal complex, thalamus and amygdala (Mesulam, 1995). Animals given toxic doses of OPs have neuropathological lesions characterized by axonal degeneration in these regions of the

brain. 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). In addition, 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. These brain regions are associated with new learning and memory, arousal, attention, executive function, response speed and emotional regulation, the cognitive functions found to be impaired in the current study of farm workers exposed to low levels of organophosphate pesticides.

The findings from this study meet Bradford-Hill criteria but before concluding that a relationship exists between low level exposure to OPs and impaired neurobehavioural functioning; and number of other potential explanations for the weak association noted between these variables will be considered.

14.2.4.2 Power analyses

Another possible reason why few correlations were observed between indices of exposure and cognitive function may be because the study did not include a sufficient number of participants to reliably detect small effect sizes. For example, power analysis indicates a sample size of between 21- 394 per group would be needed to obtain statistical power to detect a small (r=0.2) relationship between exposure and cognitive function (alpha=.05; power =.80). This range is enormous and reflects different effect sizes reported by studies in the past (see Chapters 3 and 4). Finding a suitable number of study participants to take part in the current study, who met our strict exclusion/inclusion criteria and could be assessed within the time frame allowed by the grant awarding body meant only 127 exposed sheep farmers (67 working and 60 retired) and 78 unexposed controls (38 working and 40 retired) were included in the analysis. Clearly subject numbers are at the lower end of the range recommended by power analysis, thus limiting our ability to detect significant associations; particularly once we applied statistical correction; and particularly when considered within the context of the difficulty experienced in obtaining reliable measures of exposure.

14.2.4.3 Exposure assessment

The weak dose response relationship observed in farmers may have arisen as a result of exposure misclassification. As mentioned previously, establishing a causal link between neuropsychological impairment and exposure to neurotoxic substances is not easy as objective evidence of exposure is seldom available. Often the most that can be achieved is a rough estimate regarding level and duration of exposure based on an individual's testimony / self report regarding their exposure history (Berent & Alber, 2005). Given the limits of human memory, it is possible that the exposure information collected in this study was unreliable and that critical exposure data was not captured, thus reducing the chance of finding significant associations between exposure and psychological outcome measures.

14.2.4.4 Linear dose/response curves

Another potential explanation for the weak associations observed in this study is the possibility that the relationship between exposure to OPs and neurobehavioural functioning is non-linear. A number of researchers have questioned the assumption that dose-response relationships are always linear. 'U' shaped and inverted 'U' shaped curves have been identified and threshold effects below which health effects are not apparent, but above which symptoms develop have also been noted (Hartman, 1995; Peterson Myers et al, 2009). For example, Peterson Myers and colleagues describe how hormone disrupting chemicals can have dose-response curves in which low doses cause effects opposite to high does. Low dose can stimulate (possibly by receptor up regulation) and high dose can inhibit disease (possibly by receptor down-regulation). OPs have been reported to have hormone disrupting effects, particularly on testosterone but they also disrupt the regulation of other glands in the body (Karalliedde et al, 2001). Pancetti et al (2007) and Pope (1999) discuss the fact that OPs have a wide range of effects which go beyond that of disrupting cholinergic transmission and different toxic and non-toxic responses can follow exposure. For example, drugs derived from OPs have been used to treat Alzheimer's patients in low doses (below that which disrupts cholinergic transmission) as they can potentiate cognitive function via action on other enzymes such as acylpeptide hydrolase. Peterson Myers et al (2009) argue that regulatory toxicology ignores non-monotonicity effects and may therefore underestimate risk in many cases. Hartman (1995) describes another non-linear model in which injury to the nervous system only becomes apparent when ageing results in

depletion of neuronal reserves. Furthermore, genetic differences between individuals in their capacity to detoxify and metabolise xenobiotics may render some individuals more susceptible to the effects of certain chemicals than others, thereby compounding any dose-response relationships which may exist. Hence the secondary aims of this study: to explore the possibility that some individuals are more susceptible to the neurotoxic effects of OPs than others.

14.3 Findings in relation to secondary objectives

A secondary aim of this study was to determine whether individuals who have retired on ill health grounds constitute a particular subgroup of individuals who are more susceptible to the effects of OPs than others; and to investigate whether genetic differences in the capacity to metabolise OPs, render some individuals more vulnerable to the effects of OPs than others.

14.3.1 Is there a subgroup of individuals who are more susceptible to the effects of OPs than others who have retired from the profession?

Almost all of the studies undertaken in the past concerning the neurotoxic effects of exposure to OPs have examined individuals who are fit enough to be in employment and have not considered the possibility that those with disabling disease may have left work or work in a different or reduced capacity. The only exception to this is the study by Mackenzie Ross et al (2007) in which two thirds of study participants were farmers who had retired on ill health grounds. It is possible that the large effect sizes produced by this study were due, at least in part, to the fact that persons with disabling disease were included in the study.

The current study took account of the 'healthy worker effect' by recruiting participants who had retired on ill health grounds in an attempt to determine whether they constitute a subgroup of individuals who are more susceptible to the effects of OPs than others. No significant differences were noted in terms of the exposure history of retired and working farmers. The primary reasons for retirement given by farmers was a constellation of non-specific symptoms including chronic fatigue, headaches, cognitive impairment, muscular and joint pain, numbness and chemical sensitivity; and a quarter of these farmers attributed their symptoms to OP poisoning. The primary reason for retirement given by police workers was musculo-skeletal injury.

Although differences were found between working and retired farmers on subjective, self report measures of mental and physical health, few differences were found on objective measures of cognitive function or in terms of PON1 status. The three PON1 phenotypes were equally distributed amongst working and retired farmers and similar levels of arylesterase activity were found in each group. As such, our study findings indicate that individuals who have retired on ill health grounds are not at increased risk of suffering cognitive impairment following exposure to OPs, but they do report more physical and emotional problems which would be expected.

14.3.2 Is there a subgroup of individuals who are genetically more susceptible to the effects of OPs than others?

Regarding the question of whether genetic differences in the capacity to metabolise OPs render some individuals more vulnerable to the effects of OPs than others, analysis of PON1 status was undertaken. Paraoxonase (PON1) is an enzyme which metabolises a number of organophosphate pesticides and genetic polymorphisms of PON1 have been identified in the coding sequence of PON1 (R192Q) which differ greatly in their activity towards different organophosphate pesticides and modulate the toxicity of OPs. The R 192 isoform metabolises diazoxon (the toxic metabolite of diazinon, an OP used in sheep dip in the UK) more slowly than the Q192 isoform. Cherry et al (2002 and 2011) found that the PON1 R192 polymorphism was more common in sheep farmers reporting ill health than those who are healthy and in those who report 'dippers flu'; and ill farmers were more likely than healthy farmers to have low serum hydrolytic activity for diazoxon.

Findings from the current study indicate that that there were no poor metabolisers in the exposed cohort and no relationships were found between PON1 activity levels neurobehavioural and/or health outcomes, possibly because our distribution/range of ARElase values was restricted. However, analysis by phenotype revealed lower PON1 activity in the RR group and individuals with the RR isoform were more likely to fall into the lowest quintile of performance on cognitive tests, particularly working and visual memory and reasoning tasks; and were more likely to complain of physical symptoms associated with exposure to OPs. The number of individuals identified with the RR isoform in the farming cohort was small (n=10) and so this finding needs further exploration. However, it is consistent with findings from previous research and suggests

individuals with the RR isoform may represent a vulnerable subgroup of farmers at increased risk of developing ill health following exposure to OPs.

14.4 Limitations of the current study / potential bias

The empirical study contained in this thesis has several potential weaknesses which should be considered when interpreting the results.

14.4.1 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 then it is impossible to make accurate predictions about the population as a whole. Over fifteen thousand farmers are listed on the UK Wool Marketing Board Database. As with any epidemiological study, it was only possible to examine a relatively small number of participants and it is difficult to determine how representative they are of the farming community as a whole.

A number of different recruitment methods were used in this study, including written correspondence, telephone contact and advertising, which could have resulted in selection bias and differences between working and retired cohorts. For example, most of the retired farmers volunteered to take part in the study after reading about it in an advertisement and those with neurobehavioural symptoms would be more likely / motivated to take part in the study than working farmers recruited via purposive sampling methods. This may mean the findings from this study overestimate the risk associated with exposure to OPs. Having said that, our study findings indicate that individuals who have retired on ill health grounds are not at increased risk of suffering cognitive impairment following exposure to OPs, although they do report more physical and emotional problems, which one would expect. In contrast, it is also possible that the study findings underestimate the risk associated with exposure to OPs because over 60% of potential participants had to be excluded from the study. It is therefore possible that individuals with disabling disease who are particularly vulnerable to the neurotoxic effects of OPs were inadvertently excluded as a result of the strict inclusion/exclusion criteria used.

This thesis also considered the possibility that the control group selected introduced bias by differing from farmers in some important way that exaggerated group differences.

However, this is unlikely to be the case as re-analysis of the data utilising test norms derived from a cross section of the population produced similar findings.

14.4.2 Sample size

As mentioned previously, the number of participants in the current study was relatively small and subject numbers were at the lower end of the range recommended by power analysis, thus limiting our ability to detect significant associations. Nevertheless weak associations were found between indices of exposure and predicted cognitive functions and significant differences were noted between exposed and unexposed study participants on neurobehavioural measures and physical health ratings which were consistent with study hypotheses and previous research.

14.4.3 Recall bias

As mentioned on a number of occasions, obtaining a reliable estimate of exposure is fraught with difficulty but failure to do so may result in exposure misclassification. In the current study exposure history was estimated via self-report and the accuracy of this information is open to question because self report may be distorted by inaccuracies of memory and response bias (e.g. a tendency to over or underestimate). Another 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. Having said that, study participants were unaware of study hypotheses, yet they showed a specific pattern of cognitive deficit which they could not possibly have predicted and replicated. In addition, subjects were unaware of their PON1 status at the time of assessment and were unlikely to be aware that previous research has shown that individuals with the R isoform are at greater risk of developing ill health than other isoforms, yet this finding was supported in the current study.

In an ideal world a prospective cohort study would allow for more precise quantification of exposure, but would 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 can be difficult when studying 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 (c.f. Mackenzie Ross, 2006 thesis).

14.4.4 Association is not causation

Although group and correlation analyses indicate an association exists between exposure to OPs and impaired neurobehavioural function, this does not constitute evidence of causation. Differences between exposed and unexposed cohorts may have been caused by extraneous variables which this study failed to control for; although further analyses utilising an alternative comparison group (normative data) suggests this is unlikely to be the case. It is well known in science that correlation does not imply causation but it does indicate possible causes. Austin Bradford-Hill, a medical statistician, devised criteria to assist researchers in determining whether significant results are due to real biological effects rather than random chance and this study fulfils those criteria. Hill's work and that of others, gave rise to the 'precautionary principle' which states that "when an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically" (Klaassen, 2008).

14.5 Study strengths

The main strengths of this study include:

14.5.1 Neuropsychological assessment

This study involved detailed neuropsychological assessment which is considered by many researchers to be the most sensitive means of examining the effects of toxic exposure as they reveal more regarding sub-clinical effects than internal dose indicators such as levels of toxins in blood or urine (Berent & Albers, 2005; Hartman, 1995; Lezak, 2004). Indeed, many toxins are metabolised and excreted quickly in the human body and may not leave biological markers to prove exposure or allow level of exposure to be determined. Hence, neuropsychological testing is a useful diagnostic tool in the assessment of exposed persons. This study allowed the nature and extent of neurobehavioural problems to be explored in considerable depth, using clinically sensitive measures rather than administering brief screening tests or research tools which may lack sensitivity and/or specificity. The psychometric test battery was designed to cover a range of cognitive functions and included tests which are routinely used in clinical practice for diagnostic purposes. Farmers 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 which do not cover all classes of cognitive function

14.5.2 Consideration of the 'healthy worker effect'

This was one of the few studies undertaken which took account of the 'healthy worker' effect by including individuals who had retired from work on ill health grounds. Previous studies have focused on individuals who are fit enough to work and may have underestimated risk.

14.5.3 Consideration of potentially confounding variables

This study considered a number of potentially confounding variables which could be possible causes of the outcome. For example, individuals with a past medical and psychiatric history that could otherwise account for ill health were excluded; as were individuals who have a history of acute exposure, but information was obtained about possible symptoms of undiagnosed acute exposure 'dippers flu' so that this could be considered in the analysis. The potentially confounding effects of mood state on

cognitive function were explored as was the possibility that an inappropriate control group had been selected which differed from farmers in some important way, thus exaggerating group differences. None of these variables were found to account for the cognitive deficits identified in farmers.

14.5.4 Measurement of possible vulnerability factors

This study considered the possibility that genetic differences in the capacity to metabolise OPs may render some individuals more sensitive to the neurotoxic effects of OPs than others. To this end PON1 status of study participants was determined and results indicate that individuals who carry the R isoform may be at increased risk of developing ill health following exposure to OPs.

14.6 Summary

A range of cognitive, emotional and physical problems were identified in agricultural workers with a history of low level exposure to organophosphate sheep dip. Both correlation and group analyses suggest a relationship exists between low level exposure to organophosphates and impaired neurobehavioural functioning. Few differences were found between working and retired farmers on neuropsychological tests which suggest those that have retired on ill health grounds are not a uniquely vulnerable subgroup. The cognitive deficits identified in the exposed cohort are specific and limited to response speed, working, verbal and visual memory, mental flexibility and fine motor control. They cannot be attributed to the potentially confounding effects of mood disorder, malingering, or undiagnosed acute exposure; nor have they arisen because an inappropriate control group was selected. The pattern of cognitive deficits and physical symptoms reported are consistent with reports from previous neuropsychological studies of UK sheep farmers; and this study identified a possible subgroup of individuals at increased risk of developing ill health following exposure to OPs because of a genetic difference in the capacity to metabolise OPs. The latter finding is also consistent with previous research. It therefore seems reasonable to conclude that long-term, low level exposure to OPs is associated with the development of chronic ill health involving physical symptoms, cognitive impairment and mood disorder.

14.7 Implications

The present findings suggest OP pesticides are more harmful than previously thought, even at low levels of exposure. This has implications for working practice and policies and guidelines about the use of organophosphate chemicals and for other occupational groups who are exposed to organophosphate chemicals on a regular basis, such as military personnel and aviation workers.

Follow-up studies are needed to determine whether symptoms persist, improve or worsen. At present, there are no treatment protocols for individuals who report chronic ill health following exposure to OPs and there is a need for prospective treatment trials.

It is also important to consider the possibility that clear cut dose-response relationships that might be discernable following acute exposure may not be apparent with low level exposure. Low level exposure may produce subclinical neurological injury that accumulates over time and only becomes apparent when specialised neuropsychological or neurological tests are used to evaluate patients or when neuronal reserves are depleted by processes such as ageing, thus unmasking deficits (Hartman, 1995).

The findings reported in this thesis also have implications for toxicological testing and risk assessment as they illustrate the importance of considering behavioural outcomes in addition to more traditional outcomes such as mortality, organ damage, cancers and reproductive effects. It is also important for future researchers to consider the possibility that dose-response curves may not be linear in all cases and that a host of factors may explain this from characteristics of the chemical under investigation to characteristics of the organism exposed to the chemical, such as genetic differences in the capacity to metabolise xenobiotics.

> *"All scientific work is incomplete –whether it be observational or experimental. All scientific work is liable to be upset or modified by advancing knowledge. That does not confer upon us a freedom to ignore the knowledge we already have or postpone the action that it appears to demand at a given time" (Bradford-Hill, 1965)*

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APPENDICES

APPENDIX 1: ARTICLES CONSIDERED IN THE LITERATURE REVIEW

Database Searches

Relevant studies from the 1960s onwards were identified from MEDLINE, EMBASE and PsycINFO databases (via Ovid interface) using both subject headings and textword search strategies on the 27th August 2009.

Subject heading searches were undertaken first to determine appropriate search terms for the different databases. These varied considerably, for example, in MEDLINE articles concerning 'organophosphates' are indexed under the following subject headings: 'phosphoric acid esters', 'organophosphate compounds', 'pesticides' and 'insecticides'. In PsycINFO they are indexed under the term 'insecticides' and in EMBASE as 'organophosphate', 'organophosphate pesticide' or 'organophosphate insecticide'. Relevant psychological search terms also varied considerably between databases. Terms were exploded during the searches to ensure the maximum number of potentially relevant articles were retrieved.

Textword searches were undertaken using synonyms identified from the subject heading searches, alternate spellings and truncation. Search terms were combined using BOOLEAN operators (and, or) and searches were refined by limiting them to studies of human, adult populations and studies published in the English language. For MEDLINE, the following search terms were used: organophosph* or phosphoric acid ester* or insecticide* or pesticide* and neuropsycholog* or neurobevio* or behavio* or cognit* or psychiatr* or psycholog* or anx* or depress* or memory*. For PsycINFO the terms included insecticide* and neuropsychol* or neuropsychiat* or cognit* or anx* or depress*; and for EMBASE the terms were organophosph* and neuropsychol* or neuropsychiat* or cognit* or anx* or depress*.

Details of included/excluded studies

The first step of the review process was to determine whether all 43 articles selected from the initial screening of titles and abstracts, met inclusion criteria for this review. This was not always apparent from a review of titles and abstracts. Fourteen studies were excluded following this second stage of the review because they did not meet the inclusion criteria listed in Table 1. Five studies were excluded because the information provided on exposure history was so brief, it was difficult to determine one or more of the following factors; whether the study concerned low level or high level exposure,

whether individuals with a history of acute poisoning had been included, whether individuals had been exposed to OPs as opposed to some other pesticides or the type of OPs's individuals had been exposed to was unclear (Bosma et al 2000; Dimich-Ward et al 1996; Korsak & Sato1977; Kurlycheck & Morrow 1989; Ahmed & Davies 1997). Another two studies were excluded because they involved case studies of patients who had been referred for medical evaluation and the exposure and occupational histories of the cases were extremely variable and not comparable (Richter et al 1992; Kilburn, 1999). Another four studies were excluded because they did not concern neurobehavioural outcomes following low level exposure; three concerned the association between physical symptoms and cholinesterase levels in farm workers (Ciesielski et al1994; Smit et al 2003, Ohayo-Mitoko et al 2000); and one concerned neurophysiological changes following exposure to sheep dip (Beach et al 1996). Two studies were excluded because they did not include objective measures of neurobehavioural function (Davies et al 1999; Solomon et al 2007). The study by Cox et al (2005) was excluded because it involved evaluations of children as well as adults following domestic exposure to methyl parathion. The findings from adults and children were not presented separately.

Number of potentially relevant articles identified during initial literature searches

Database	Number of potentially relevant articles identified	Initial exclusions	Number deemed potentially relevant following Title & Abstract review
MEDLINE			
Subject Heading search	186	169	17
Textword search	184	159	25
PsycINFO			
Subject Heading search	27	23	4
Textword search	21	17	4
EMBASE			
Subject Heading search	49	38	11
Textword search	95	77	18
Totals	562	483	79

Articles selected for further review

	AUTHOR	ML - KEY	ML - TXT	PI - KEY	PI-TXT	EB-KEY	EB-TXT
1	Ahmed & Davies 1997					X	X
2	Albers et al, 2004		X				X
3	Ames et al, 1995	X	X				X
4	Bazylewicz-Walczak et al, 1999		X				
5	Beach et al, 1996		X				X
6	Besseler et al, 2006			X	X		
7	Bosma et al, 2000	X					
8	Ciesielski et al, 1994						X
9	Cole et al, 1997		X	X	X	X	X
10	Cox et al, 2005						X
11	Daniel et al, 1992	X	X				
12	Davies et al, 1999					X	X
13	Dimich-Ward et al, 1996	X					
14	Farahat et al, 2003	X	X				
15	Fiedler et al, 1997	X	X				
16	Jamal et al 2001	X	X			X	X
17	Kamel et al, 2003	X					
18	Kamel et al, 2007		X				
19	Kilburn 1999						X
20	Korsak & Sato 1977		X				
21	London et al, 1997		X				
22	Maizlish et al, 1987	X	X				
23	Misra et al, 1994		X				X
24	Parron et al, 1996					X	X

25	Richter et al, 1992		X				
26	Rodnitzky et al, 1975		X				
27	Rohlman et al, 2007	X	X				X
28	Roldan-Tapia et al, 2005	X	X		X		
29	Roldan-Tapia et al, 2006	X	X	X	X	X	X
30	Rothlien et al, 2006					X	X
31	Salvi et al, 2003	X	X			X	X
32	Srivastava et al, 2000	X	X				
33	Steenland et al, 2000		X			X	
34	Stephens et al, 1995	X	X			X	X
35	Stephens et al, 1996	X	X	X			
36	Stephens & Sreenivasan, 2004	X	X			X	X
Additions							
37	Mackenzie Ross et al, 2007						
38	Browne et al, 2006						
39	Ohayo-Mitoko et al, 2000						
40	Smit et al, 2003						
41	Solomon et al, 2007						
42	Kurlycheck & Morrow 1989						
43	Amr et al, 1997						

Neurobehavioural tests used by previous researchers and the cognitive domains they were assigned to for the purpose of meta-analysis.

Cognitive domain	**Tests**
Working memory & attention	Digit Span Tests
Psychomotor speed	Digit symbol Trails A Reaction Time simple AMIPB Speed Hand/eye co-ordination Tapping
Attention & vigilance	Vigilance Paced Auditory Serial Addition Test Letter cancellation AMIPB Task A Sustained attention Continuous performance
Verbal – hold tests	Vocabulary Reading Naming Token Test Sentence WAIS VIQ
Executive function	Similarities Trails B Syntactic reasoning Verbal fluency Reaction time – choice Stroop
Visuo-spatial	Block design Line orientation Benton Visual Form Test WAIS PIQ

Visual memory	Benton Visual Retention Test Pattern memory Face recognition Picture completion Rey Ostereith [ROC] AMIPB figure recall AMIPB design learning Figure Recall Location recognition
Verbal memory	Story recall or Logical Memory Auditory Verbal Learning Test California Verbal Learning Test List learning Serial digit Category learning
Mood	Anxiety measures Depression measures
Fine motor control	Santa Ana Manual Dexterity Test Pursuit Aiming Grooved Pegboard

AMIPB = Adult Memory and Information Processing Battery; WAIS = Wechsler Adult Intelligence Scale.

APPENDIX 2 – MEASURES

Exposure Questionnaire

Physical Health Questionnaire

Exposure questionnaire

Name / Code No: _____

DOB: _____

TEL: _____

A.Occupation

1) Which of the following best describes your occupation (tick)?

Farm Owner __
Farm Tenant __
Farm Manager __
Farm Worker __
Sheep dip contractor __
Other (state) _____

2) So are / were you
- employed __ ?
- self-employed __ ?

3) How many years in farming? _____

4) Are you
- working __ ?
- retired __ ?
- semi-retired __ ?
- Changed occupation __ ?

B. Type of work carried out whilst farming (circle number)

	TASK	***Go to***
1	Sheep dipping	C
2	Shearing / Handling recently dipped (within 3 weeks) sheep / fleeces	D
3	Treating cattle for warble fly / handle treated cattle	E
4	Crop / weed spraying	F
5	Treating grain or working with treated grain	G
6	Working in orchards	H
7	Domestic use of pesticides *1) Headlice treatment* *2) Pet treatment* *3) Fly killer* *4) Fumigation* *5) Timber treatment* 6) *Other - specify*	I
8	Other work with pesticides	J
9	Using solvents (excluding those in sheep dip)	K
10	Using lead	L
11	Using vibrating equipment	M

C. Sheep Dipping

				Go to
Time scale and extent of exposure				
1	When was the last time you were exposed to sheep dip product?			2
2	Have you ever applied sheep dip product using sprays or showers?	Yes __ State which: No __		3 4
3	Over what years?	State _____		4
4	Between what years have you been involved in dipping sheep?			5
5	How many times each year?	State years if variation:		6
6	For how many days each time?	State years if variation:		7
7	For how many hours each day?	State years if variation:		8
8	What was the average flock size (inc. lambs)?	State years if variation:		9
9	Whilst sheep dipping, what was your main role? (circle)	(a) Dunking / plunging (b) Chucking (c) Dry herding (d) Wet herding (e) Mixing concentrate		10
10	Did you ever do any of the following tasks not circled in 9.?	(a) Dunking / plunging (b) Chucking (c) Dry herding (d) Wet herding (e) Mixing concentrate		11
11	Which of the following did you most regularly use to submerge the sheep?	(a) Implement __ (b) Hands __ (c) Feet __ (d) Other (state) __ _____		12
12	Did you ever have to go into the dipping bath, e.g. to rescue sheep?	Yes __ No __	Years: _____	13

Accidents				
13	How often did you get splashed with sheep dip on any part of the body? (circle)	(a) Always (b) Usually (c) Sometimes (d) Never		14 15
14	Where did you get splashed most frequently? (circle)	(a) Hands (b) Arms (c) Feet (d) Legs (e) Face / Neck (f) Head (g) Torso / upper body		15
15	How often did you get soaked to the skin on any part of the body? (circle)	(a) Always (b) Usually (c) Sometimes (d) Never		16 17
16	Where did you get soaked to the skin most frequently? (circle)	(a) Hands (b) Arms (c) Feet (d) Legs (e) Face / Neck (f) Head (g) Torso / upper body		17
17	When did you wash the splashed or soaked areas of skin?	Area (a-g) (a) Immediately (b) End of dipping (c) Before breaks - meals - smoking (d) Other - specify _____		18
18	What washing facilities were used?	(a) Open tank (b) Bucket (c) Hosepipe (d) Cold running water (e) Hot running water (f) Shower (g) Soap (h) Other specify:		19
19	Have you ever fallen into the dipping bath?	Yes __ No __		20 21
20	When did you fall in?	Years:		21

Health following exposure				
21	Did you ever suffer from 'Dippers Flu' after dipping sheep?	Yes __ No __		22 23
22	How often did you suffer from 'Dippers Flu'?	Frequency:		22
23	Did you ever feel any other ill health symptoms following sheep dipping?	Yes __ No __		24 25
24	What were these symptoms and how often did you suffer from them?	Symptoms: 1) 2) 3) 4) 5)	Frequency:	25
25	Did you ever have to seek medical help after dipping?	Yes __ No __		26 28
26	With whom did you seek medical help?	(a) Local GP __ (b) Local Nurse __ (c) Hospital A&E __ (d) Specialist __ (e) Other __ *Please state:* _____		27
27	How soon afterwards after each symptom appeared did you seek medical help?	Symptom: (a) < 24 hours (b) < 48 hours (c) < 72 hours (d) < 1 week (e) < 2 weeks	Frequency:	28
Sheep dipping area and dipping method				
28	Location of bath?	(a) Within building (b) Within mobile trailer (c) Outside exposed (d) Outside sheltered (e) Covered (open sides) (f) Other state_____	Years:	29
29	Was there a screen to deflect splashes across	(a) Dip bath entry (b) Sides of dip bath (c) Dip bath exit	Years:	30
30	How many times per day did you have to replenish the bath?	(a) 0 (b) 1 (c) 2 (d) 3+		31

Sheep dip concentrate				
31	Did you work with sheep dip concentrate?	Yes __ No __		32 38
32	Approximately how much concentrate did you normally use in a single dipping session?	(Specify pints/litres/gallons) Name: amount: Name: amount: Name: amount:	Years:	33
33	Can you remember the strength (% active ingredient in) the pesticide concentrate you used? *(or write in 'strong' / 'weak')*	% % %	Years:	34
34	How often did you get splashed with sheep dip concentrate on any part of the body? (circle)	(a) Always (b) Usually (c) Sometimes (d) Never		35 38
35	If any of (a)-(c) above, how often did you wash off the concentrate?	(a) Always (b) Usually (c) Sometimes (d) Never		36
36	How soon after the spillage?	(a) Immediately (b) Within minutes (c) Within hours		37
37	On average, how many times per dipping period were you involved in any accidents (e.g. spillage) with concentrate?	(a) 0 (b) 1 (c) 2 (d) 3+		38
Maintenance of the dip				
38	Did you use meter systems to transfer concentrate to the bath?	Yes __ No __		39
39	Have you ever been involved in emptying the dipping bath?	Yes __ No __		40
40	How did you empty the bath?	(a) Slurry tanker (b) Pails (c) Other state _____		41
41	How often have you been involved in cleaning the dipping bath?	(a) Never (b) Once (c) 2-3 times (d) Other frequency – state: _____ (e) Every dipping session		42

46) Which of the following protective clothing and safety equipment did you use when both sheep dipping and using concentrate? (For each circled item, ask i-iv)

Protective clothing / safety equipment (circle)	i) Years used	ii) For which specific tasks?	iii) What % of the day did you use the item?	iv) What type of material was the item made of? (e.g. Rubber, nitrile, plastic, leather)
Sheep dipping				
(a) Waterproof trousers / waders / leggings				
(b) Waterproof footwear				
(c) Waterproof overalls				
(d) Waterproof jacket				
(e) Waterproof gloves				
(f) Visor / face shield				
(g) Hat				
(h) Bib / Apron				
(i) None				
Concentrate				
(a) Waterproof trousers				
(b) Waterproof footwear				
(c) Waterproof overalls				
(d) Waterproof jacket				
(e) Waterproof gloves				
(f) Visor / face shield				
(g) Hat				
(h) Bib / Apron				
(i) None				

47) Please read the following list of sheep dip products and place a tick next to the products you have previously used, and between what dates.

PRODUCT NAME	Have you used this product?		YEARS USED
	YES	NO	

D. Shearing / Handling recently dipped sheep / fleeces

				Go to
1	Have you ever worked with	(a) Recently dipped sheep (b) Fleeces of recently dipped sheep		2
2	Between what years?			3
3	How soon after the sheep had been dipped were you handling them?			4
4	What activity were you doing with the sheep or fleeces?	(a) Shearing (b) Handling fleeces (c) Inspecting Sheep (d) Other. State:_____		5
5	What protective clothing did you wear whilst handling the dipped sheep, and over how many years?	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	7 7 7 7 **6** 7 7 7 7
6	What type of gloves were they?	(a) rubber (b) nitrile (c) plastic (d) leather (e) Other. State:_____		7
7	Approximately how many hours did each contact last for?			8
8	How many years did you do this for?			9
9	How many times a year did you do this activity?			End

E. Treating cattle for warble fly / handle treated cattle

				Go to
1	Between what years did you use pesticide to treat warble fly on cattle?			2
2	How many times per year?			3
3	Over how many days?			4
4	How was the product applied to the cattle?	(a) Dip (b) Spray (c) Pour on (d) Other (specify)		5
5	What protective clothing did you wear whilst using the treatment and over how many years?	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	6
6	Did you dilute the pesticide?	Yes __ No __		7
7	Was the chemical used an organophosphate pesticide?	Yes __ No __		8
8	What were the names of the pesticides you used?			9
9	Did you handle the treated cattle?	Yes __ No __		10 End
10	How many days per year did you work with the treated cattle?			11
11	How many hours per day?			End

F. Crop / weed spraying

				Go to
1	Between what years did you apply pesticide to arable crops, fodder crops or grassland?			2
2	How many times per year?			3
3	Over how many days?			4
4	How was the pesticide applied? (circle)	(a) Aerial spray (b) Tractor spray (c) Other (specify) _____		5
5	What protective clothing did you wear whilst using the pestcide and over how many years? (circle)	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	6
6	Did you dilute the pesticide?	Yes __ No __		7
7	Was the chemical used an organophosphate pesticide?	Yes __ No __		8
8	What were the names of the pesticides you used?			9
9	Did you handle the treated crops or enter the treated fields?	Yes __ No __	Details:	End

G. Treating grain or working with treated grain

				Go to
1	Between what years did you use insecticide to treat grain?			2
2	How many times per year?			3
3	Over how many days?			4
4	How was the insecticide applied? (circle)	(a) Spray (b) Smoke bomb (c) Other (specify) _____		5
5	What protective clothing did you wear whilst using the insecticide and over how many years? (circle)	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	6
6	Did you dilute the insecticide?	Yes __ No __		7
7	Was the chemical used an organophosphate?	Yes __ No __		8
8	What were the names of the insecticides you used?			9
9	How soon after treatment did you enter the store? (Specify)	_____ hrs / days		10
10	In the 2 weeks following the treatment, how many days did you work there?			End

H. Working in orchards

				Go to
1	Between what years did you use pesticides in orchards?			2
2	How many times per year?			3
3	Over how many days?			4
4	How was the pesticide applied? (circle)	(a) Spray (b) Tractor (c) Other (specify) _____		5
5	What protective clothing did you wear whilst using the insecticide and over how many years? (circle)	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	6
6	Did you dilute the pesticide?	Yes __ No __		7
7	Was the chemical used an organophosphate?	Yes __ No __		8
8	What were the names of the pesticides you used?			9
9	How soon after it had been sprayed did you pick the fruit? (Specify)	_____ hrs / days		10
10	How many days did you spend picking the treated fruit?			End

I. Domestic use of pesticides

	(1) Head lice treatment	(2) Pet treatment	(3) Fly killer	(4) Fumigation	(5) Timber treatment	(6) Other - specify: _____
a) Have you ever used (1 to 6) **If yes, a–f below**	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __
b) Between what years?						
c) How many time per year?						
d) Did it contain organophosphate?	Yes __ No __ Don't know __	Yes __ No __ Don't know __	Yes __ No __ Don't know __	Yes __ No __ Don't know __	Yes __ No __ Don't know __	Yes __ No __ Don't know __
e) How did you handle the product? E.g. how applied and protection used						
f) Did you dilute the product?	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __	Yes __ No __
g) What was the name of the product?						

J. Other work with pesticides

				Go to
1	For what purpose were the pesticides used?			2
2	Over how many years?			3
3	How many times per year?			4
4	How many hours per day?			5
5	How was the pesticide applied? (circle)	Specify _____		6
6	What protective clothing did you wear and over how many years? (circle)	(a) Waterproof trousers (b) Waterproof footwear (c) Waterproof overalls (d) Waterproof jacket (e) Waterproof gloves (f) Visor / face shield (g) Hat (h) Bib / Apron (i) None	Yrs Yrs Yrs Yrs Yrs Yrs Yrs Yrs ---	7
7	Did you dilute the pesticide?	Yes __ No __		8
8	Was the chemical used an organophosphate?	Yes __ No __		9
9	What were the names of the pesticides you used?			End

K. Using Solvents

				Go to
1	For what purpose were the solvents used?			2
2	Over how many years?			3
3	How many times per year?			4
4	How many hours per day?			5
5	How was the solvent applied? (circle)	Specify _____		6
6	Did you wear either of the following? And over what years?	(a) Gloves (b) respirator	Yrs Yrs	7
7	How much solvent did you and others working near to you use each day?	(a) <50ml (<1.75 floz) (b) 50-500 ml (1.76-17.6 floz) (c) 501-2000 (17.7 floz–3.5 pints) (d) > 2 litres (>3.5 pints)		8
8	Did you do this work in	(a) a large well ventilated room (b) a large poorly ventilated room (c) a small well ventilated room (d) a small poorly ventilated room (e) outside		9
9	Were there any measures in the workplace to control your exposure to solvents?	Yes __ No __		10
10	What were the names of the solvents you used?			End

L. Using Lead

				Go to
1	For what purpose was the lead used?			2
2	Over how many years?			3
3	How many times per year?			4
4	How many hours per day?			5
5	Did you wear either of the following? And over what years?	(a) Gloves (b) respirator	Yrs Yrs	6
6	Was the lead heated in the process?	(a) Yes (b) No (c) Don't know		7
7	Did you do this work in	(a) a large well ventilated room (b) a large poorly ventilated room (c) a small well ventilated room (d) a small poorly ventilated room (e) outside		8
8	Were there any measures in the workplace to control your exposure to solvents?	Yes __ No __		9
9	What were the names of the lead products you used?			End

M. Using vibrating equipment

				Go to
1	What type of equipment / tool did you use?			2
2	For what purpose was the tool / equipment used?			3
3	Over how many years?			4
4	How many times per year?			5
5	How many hours per day?			6
6	Was the tool very noisy?	(a) Yes (b) No		7
7	What was the source of vibration?			8
8	Were you sitting or standing on the vibrating surface?	(a) Sitting (b) Standing		9
9	Were there any measures in the workplace to control your exposure to vibrating equipment?	Yes __ No __		End

Physical Health

This questionnaire asks for your views about your health. It asks how you feel and how well you are able to do your usual activities.

1. In general, would you say your health is: (Please tick **one** box)

Excellent ☐ 1

Very Good ☐ 2

Good ☐ 3

Fair ☐ 4

Poor ☐ 5

2. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**? (Please circle Yes or No)

2a. Reduced the **amount of time** you spent on work or other activities **Yes** **No**

2b. Accomplished less than you would like **Yes** **No**

2c. Were **limited** in the **kind** of work or other activities **Yes** **No**

2d. Had **difficulty** performing the work or other activities (for example, it took extra effort) **Yes** **No**

3. During the **past 4 weeks,** to what extent has your physical health interfered with your normal social activities with family, friends, neighbours, groups etc? (Please tick **one** box)

Not at all ☐ 0

Slightly ☐ 1

Moderately ☐ 2

Quite a bit ☐ 3

Extremely ☐ 4

4. How much **bodily pain** have you had in the **last 4 weeks?** (Please tick **one** box**)**

None	☐ 0	Moderate	☐ 3
Very mild	☐ 1	Severe	☐ 4
Mild	☐ 2	Very Severe	☐ 5

5. During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and household tasks)? (Please tick **one** box**)**

Not at all	☐ 0	Quite a bit	☐ 3
A little bit	☐ 1	Extremely	☐ 4
Moderately	☐ 2		

6. **Physical symptoms:** During the last **4 weeks** have you suffered from any of the following symptoms? (Please circle a response in the column headed How often?)

   In addition, for those symptoms that you have experienced, please tell us when the symptom first appeared and indicate how severe and how distressing you find each symptom by circling the most appropriate responses separately for each symptom.

   Please also circle a response in the final column for each symptom you have experienced to rate the impact you feel it has had on your ability to carry out your normal daily activities.

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Problems controlling temperature/sweating	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Headaches	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Toothaches	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Loss of sensation in fingers and toes	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Blurred vision	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Numbness or tingling in any part of the body (Please state where _____ _____ _____)	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Fatigue	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Loss of balance/co-ordination	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Dizziness	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Problems with sexual functioning (e.g. impotence, loss of interest in sex)	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Ringing in ears	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Skin complaints	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Temporary deafness or hard of hearing	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Joint stiffness or pain (Please state where) _____ _____ _____ _____	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Sleep problems (difficulty sleeping or too much sleep, please elaborate)_____ _____ _____	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Muscle tremors	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Nausea	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Swollen glands	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Pain in hands or feet	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Difficulty remembering/concentrating	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Dry mouth	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Chest pains/tightness	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Feeling disorientated	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Muscle weakness (Please state where _____ _____ _____)	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Burning sensation (Please state where _____ _____ _____)	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Shortness of breath	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Decrease in sensitivity to touch/pain/temperature	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Feeling slowed down (mentally)	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Feeling feverish	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Unintended weight loss or gain	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Gastro-intestinal problems (Please elaborate)_____ _____ _____	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Diarrhoea/Constipation	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Hay fever or allergies	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Irritability/temper control problems	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Coughing	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Cramps (Please state where) _____ _____ _____ _____	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

Symptom	How often?	Date symptom first appeared?	How severe?	How distressing do you find this symptom?	Does it interfere with daily activities? (for example housekeeping, work, leisure)
Muscle twitching	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Chemical/alcohol intolerance	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time
Urinary problems	0 Never 1 Sometimes 2 Usually 3 All of the time		1 Mild 2 Moderate 3 Severe	0 Not at all 1 A little 2 Moderately 3 Extremely	0 No 1 Sometimes 2 Usually 3 All the time

If there are any symptoms not covered above please use the space below to tell us about them. Please also give any further details about any of the symptoms above that you would like to add:

APPENDIX 3 – ETHICAL APPROVAL