Appendix 1
Glossary and Abbreviations

Acceptable daily intake	The acceptable daily intake is defined as 'the amount of a chemical which can be consumed every day of an individual's entire lifetime in the practical certainty, on the basis of all known facts, that no harm will result'
Acetylcholinesterase	Enzyme responsible for the hydrolysis of acetylcholine in nerve and muscle tissue, and also present in other tissues such as red blood cells. It splits acetylcholine into acetic acid and choline
AChE	see Acetylcholinesterase
ACP	Advisory Committee on Pesticides
ACT	Automated cognitive testing system
Acute toxicity	In the context of this report acute toxicity is used to describe effects that occur over a short period of time (hours or a few days) immediately following exposure
ADAS	Agricultural Development and Advisory Service
ADI	see Acceptable daily intake
Adipose tissue	Connective tissue containing fat cells
AFQT	Armed Forces Qualifying Test
Ageing	Of acetylcholinesterase, a chemical change within the organophosphorylated form of inhibited acetylcholinesterase resulting in a stable form of the inhibited enzyme, which cannot then reactivate
Anoxia	A condition in which tissues receive inadequate oxygen
Anticholinesterase	A substance that inhibits the action of cholinesterase enzymes
AOEL	Acceptable Operator Exposure Level
Aphasia	A disorder of language affecting the production and understanding of speech
Attention	The ability to concentrate on a single source of sensory information or to resist distraction while carrying out a specific task
Ataxia	Loss of coordination of the limbs leading to irregular and uncontrolled limb movements
Autonomic nervous system	The part of the nervous system responsible for the control of body functions that are not directed consciously

Organophosphates	**Biomarker**	Any substance, structure or process that can be measured in the human body or its products and may influence or predict the incidence or outcome of disease. Biomarkers can be broadly classified into markers of exposure, effect or susceptibility
	Blinding	Steps taken to ensure that an investigator or subject is unaware of information that could bias the assessment of a variable measured in an epidemiological study, e.g. keeping the investigator who is evaluating the health of a subject ignorant about the exposure of that subject
	BVA	British Veterinary Association
	Cadaveric	Taken from dead bodies
	CFOC	Canadian Farm Operatives Cohort
	Cholinesterase	An enzyme that breaks down a choline ester into its choline and acid components
	Chronic toxicity	In the context of this report chronic toxicity is used to describe effects of long duration
	CI	see Confidence Interval
	Class effects	Common effects brought about by each member of a group of compounds that act by similar mechanisms
	cMAP	see Compound muscle action potential
	CMO	Chief Medical Officer
	CNS	Central nervous system
	Cognitive	Relating to higher mental functions such as memory, attention and language
	Cold threshold	(Cooling threshold) The smallest decrease in temperature of a device placed on the skin that is perceptible to an individual. See Quantitative sensory testing, QST
	Compound muscle action potential	The electrical response in a muscle to nerve stimulation, resulting in contraction of the muscle. Its amplitude, measured in nerve conduction tests, is an index of the number of muscle fibres in functional continuity with the nerve supply of the muscle, and may thus be abnormal in diseases of peripheral nerve or muscle
	Conduction velocity	Speed of conduction of impulses along the nerve, typically reduced in diseases causing damage to the insulating sheaths around nerve fibres (myelin sheath). There is much less slowing if nerve damage is restricted to the central fibres (axons)

Confidence interval	Confidence Interval, specified as 95%CI or 90%CI, the range of values within which there is a 95% or 90% chance respectively of the true result falling
COPIND	Chronic OP-Induced Neuropsychiatric Disorder
Corticospinal tracts	Bundles of nerve fibres running between the brain and the spinal cord conducting impulses from the motor cortex of the brain to the muscles
COSHH	Control Of Substances Hazardous to Health regulations applicable at work
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
Cross-sectional study	A study based on information about the prevalent exposures and/or disease in a population that is collected at one point in time
CYP2C8	Cytochrome P-450 isoenzyme 2C8, one of a family of enzymes involved in the metabolism of lipid-soluble molecules including drugs, sterols and environmental chemicals
CYP3A4	Cytochrome P-450 isoenzyme 3A4, see CYP 2C8
DEF	*S,S,S*-tributylphosphorotrithioate, an OP defoliant
DEP	Diethylphosphate, a metabolite of some OPs which is excreted in the urine
DEPT	Diethylphosphorothioate, a metabolite of some OPs which is excreted in the urine
DH	Department of Health
Distal	Situated away from the point of origin or attachment
DMP	Dimethylphosphate, a metabolite of some OPs which is excreted in the urine
DMPT	Dimethylphosphorothioate, a metabolite of some OPs which is excreted in the urine
EEG	see Electroencephalography
Effector organ	The structure that brings about activity, such as muscular contraction or glandular secretion
Electroencephalography	A technique for recording the electrical activity of the brain, used for clinical investigation of brain function
Electromyography	A technique for investigating neuromuscular function by recording electrical activity from muscles
EMG	see Electromyography

Organophosphates	**End plates**	The parts of muscle cells immediately adjacent to the motor nerve terminal. They contain the receptors for acetylcholine and are responsible for triggering muscle contraction in response to the stimulus from the motor nerves
	EPN	The recognised common name of a particular OP pesticide
	Erythrocyte	Red blood cell
	Esterases	Enzymes that break down esters into their acid and alcohol components
	GHQ	General health questionnaire
	Half-life	The time taken for a process to be half completed. It often refers to the time for the plasma concentration of a drug or chemical to fall by 50%
	HSE	Health and Safety Executive
	Hypoxic	Deficient in oxygen
	Idiopathic	Denoting a disease or condition for which the cause is not known or that arises spontaneously
	IEH	Institute for Environment and Health of the Medical Research Council
	In vitro	A Latin term used to describe effects in biological material or experiments outside the living animal
	In vivo	A Latin term used to describe effects or experiments in living animals
	IOH	Institute of Occupational Health, Birmingham
	IOM	Institute of Occupational Medicine, Edinburgh
	JEM	Job-exposure matrix
	Jitter	A measure of the variability in the delay between excitation of a motor nerve and excitation in its target muscle fibre, measured on repeated stimulation of the nerve
	Labile group	Part of a molecule which is easily removed from the remainder of the molecule by cleavage of an easily broken or 'labile' bond
	LD_{50}	The estimated dose that would result in the death of 50% of the exposed animals
	Long term memory	Memory for information acquired at a specific time and place and no longer available in short term memory
	Lymphocyte	A specific type of white blood cell involved in the body's immune response
	MAFF	Ministry of Agriculture, Fisheries and Food

MCA	Medicines Control Agency
Median nerve	One of the major motor and sensory nerves in the arm
Meta-analysis	A statistical analysis in which data from two or more different studies is formally combined in order to carry out a single significance test or calculate a single estimate. It is most commonly used to combine evidence from several different clinical trials or from several different epidemiological studies
Metabolic activation	Conversion by enzymes of a chemical from one state to another state which is biologically more active
Metabolite	A product formed from a compound by enzymic reactions in the body/cell
MMPI	Minnesota Multiphasic Personality Inventory
Motor	Relating to the output division of the nervous system carrying information from the brain to the muscles
MRC	Medical Research Council
Muscarinic sites	Regions containing receptors that respond to acetylcholine and to compounds structurally related to muscarine, a poison derived from fungi
Muscle fasciculation	Brief spontaneous contraction of a few of the fibres within a muscle, seen usually as a flicker of movement under the skin
Myasthenic	Relating to muscle weakness due to abnormal neuromuscular transmission i.e., disorder of the junction between the peripheral nerve and muscle
NCTB	Neurobehavioural core test battery
NES	Neurobehavioural evaluation system
NPIS	National Poisons Information Service
Nerve conduction studies	Technique for investigating function of peripheral nerves. (See Electromyography)
Neural	Relating to nerves
Neurobehavioural	see Neuropsychological, the preferred term in this report
Neuromuscular	Relating to the muscle, the neuromuscular junction and peripheral nerve
Neuromuscular dysfunction	Abnormal combined function of a muscle and its controlling nerve
Neurons	Nerve cells

Organophosphates	**Neuropathy target esterase**	An esterase enzyme (formerly known as neurotoxic esterase) which is associated with the development of organophosphate-induced delayed neuropathy. It was originally defined by the method of measuring its activity against an artificial substrate and selective inhibition of that activity by certain organophosphates. The protein has since been identified but the natural substrate is unknown
	Neuropsychological	Relating to the function of the central nervous system, as measured by behaviour
	NFU	National Farmers' Union
	NIOSH	National Institute of Occupational Safety and Health, a United States organisation
	NOAEL	see No observed adverse effect level
	NOAH	National Office of Animal Health Ltd
	No observed adverse effect level	The maximum observed daily dose which does not produce the effects detected at higher doses
	NPTC	National Proficiency Test Council
	NTE	see Neuropathy target esterase
	OP	Organophosphate
	OPIN	OP Information Network
	OPIDPN	Organophosphate-induced delayed polyneuropathy
	OR	Odds ratio, the ratio of the odds of an outcome occurring in each of two groups
	Paraoxonase	An esterase capable of hydrolysing a range of esters usually assayed by its ability to hydrolyse paraoxon
	Paraesthesiae	Spontaneously occurring abnormal tingling sensations
	Parasympathetic nerve	A nerve of the parasympathetic division of the autonomic nervous system. These nerves have fibres that connect the brain and spinal cord to blood vessels, glands and the majority of internal organs. They release acetylcholine at their target sites
	Pathogenesis	The biological mechanisms underlying the clinical manifestation of disease
	PEGS	Pesticide Exposure Group of Sufferers
	Peripheral baroreceptors	Sensory nerve endings specialised to monitor changes in blood pressure in blood vessels throughout the body
	Peripheral neuropathy	A disorder affecting the nerves outside the brain and spinal cord

Peroneal nerve	Major motor and sensory nerve of the lower leg, a branch of the sciatic nerve
Phagocytosis	The engulfment and digestion of particles of microorganisms by a cell
Phosphorylation	Addition of a phosphate group to a molecule
PIAP	HSE's Pesticides Incidents Appraisal Panel
Plasma	The fluid portion of blood. When plasma is clotted, the thinner fluid separating from the clot is serum
PMSF	Phenylmethanesulphonyl fluoride
PNS	Peripheral nervous system
Polymorphism	The existence of variation of a genetic characteristic in a population
POMS	Profile of mood states
Post-synaptic acetylcholine receptors	Nerve terminals pass signals to their target cells in a specialised region, known as the synapse. Structures and events occurring in the signalling cell are known as pre-synaptic, those in the target cell are post-synaptic. Post-synaptic acetylcholine receptors are small, discrete areas on the post-synaptic cell membrane which interact with acetylcholine released from the terminal of the pre-synaptic cell. This interaction initiates a change in the working of the post-synaptic cell
Potentiation	Increased activity or effectiveness of an agent as the result of the presence of another factor
PPE	Personal protective equipment
Prospective longitudinal study	(Also known as a prospective cohort study). A method of epidemiological study in which defined population is identified and then followed up over time with ascertainment of exposures and/or subsequent disease or mortality
Proteases	Enzymes which break down proteins
Proximal toxin	The form of a toxin that interacts directly with its target site
PSD	Pesticides Safety Directorate
Quantitative sensory testing	Investigative technique for studying function in sensory nerve fibres by recording the magnitude of a sensory stimulus just sufficient to be perceived by an individual. The technique examines nerve fibres not accessible to routine nerve conduction studies but is dependent on the active co-operation of the individual. See Cold threshold, Warm threshold, Vibration threshold

Organophosphates	**Radiolabel balance study**	A study in which a compound labelled with one or more radioactive isotopes is administered and the fate of all the radioactivity is monitored over time until all the material administered has been accounted for. Usually urine, faeces, expired air and tissue residues are examined
	RCVS	Royal College of Veterinary Surgeons
	Receptor	A small, discrete area on the cell membrane or within the cell with which specific molecules interact to initiate a change in the working of a cell
	Receptor activation	A change in the state of a receptor following interaction with a specific signalling molecule. The state change is the first step in the cellular response to the signal
	Regression analysis	The body of statistical theory and methods for exploring relationships between variables. A simple form frequently used in data analysis examines the linear relationship between two variables and estimates the average increase in one variable that is associated with a change of size of one unit in the other variable
	Safety factor	see Uncertainty factor
	SARSS	Human Suspected Adverse Reaction Surveillance Scheme of the Veterinary Products Committee
	SD	Standard deviation
	Semantic memory	Generic knowledge about the world not related to any one specific past event (e.g. knowing that Paris is the capital of France or what a kangaroo is, etc.)
	Sensory	Relating to initial registration and perception of external events
	Sequela	Any disorder or pathological condition that results from a preceding disease or accident
	Serum	The fluid remaining after blood has clotted
	SFEMG	see Single fibre EMG
	Signs	Abnormalities that are observed by a physician or investigator but that may not be apparent to the patient or subject
	Short term memory	Memory for information acquired only a short while (i.e. a few seconds) before testing, actively maintained by rehearsal (e.g. the kind of memory employed to remember a telephone number from a directory while dialling it)
	Single fibre EMG	Electromyographic technique; highly sensitive method of testing for disorders of neuromuscular transmission which may also be abnormal in motor neuropathies

Spasticity	Resistance to the passive movement of a limb associated with increased muscle tone. It is usually accompanied by weakness
Sural nerve	A predominantly sensory nerve in the leg
Sympathetic ganglionic autonomic nerve endings	Terminals of the sympathetic nerves of the autonomic nervous system, which leave the central nervous system and terminate in structures known as sympathetic ganglia located in a chain on either side of the spinal cord. The terminals act on nerve cells in the ganglia which in turn send fibres to blood vessels, glands and other internal organs
Symptom	An abnormality noticed by the patient or subject themselves
Synaptic	Relating to the area of contact between a nerve terminal and its target cell membrane
Synergism	Interaction of one agent with another to produce increased activity, which is greater than the sum of the effects of the two agents separately
TEPP	Tetraethyl pyrophosphate
Thermal threshold test	Test for measuring function in sensory fibres for temperature perception. (See Warm threshold, Cold threshold)
Transdermal absorption	Absorption across the skin
TOCP	Triorthocresyl phosphate
Toxicokinetics	The description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and excretion
Toxicodynamics	The description of the interaction of a chemical with its site of toxic action
UK	United Kingdom
Ulnar nerve	One of the major nerves in the arm
Uncertainty factor	The factor by which the NOAEL (*qv*) identified in the most sensitive and relevant animal or human study is divided in order to obtain the ADI (*qv*). It is used to allow for the uncertainty when extrapolating from animal data to humans, for interindividual variability in humans and also for the adequacy of the overall database and the severity of the toxic effects observed. In the past the term safety factor has been used but 'uncertainty factor' is considered to be a more appropriate expression since it avoids the notion of absolute safety and because the size of the factor is proportional to the magnitude of uncertainty rather than safety.
USA	United States of America

Organophosphates	**Vibration threshold**	Smallest vibratory sensory stimulus perceptible to individual. (See Quantitative sensory testing)
	VMD	Veterinary Medicines Directorate
	VPC	Veterinary Products Committee
	v/v	Volume/volume, to indicate that measures of volume are used in the preparation of a solution or mixture
	Warm threshold	Smallest increase in temperature of a device placed on the skin that is perceptible to an individual. (See Quantitative sensory testing, QST)
	WAIS	Wechsler Adult Intelligence Scale
	WHO	World Health Organization
	WMS	Wechsler Memory Scale
	WPPR	Working Party on Pesticide Residues
	WRAT-R	Wide Range Achievement Test – Reading

Units

mg	=	1 milligram (10^{-3}g or 1/1000 of a gram)
µg	=	1 microgram (10^{-6}g or 1/1,000,000 of a gram)
ng	=	1 nanogram (10^{-9}g or 1/1,000,000,000 of a gram)
pg	=	1 picogram (10^{-12}g or 1/1,000,000,000,000 of a gram)
ppm	=	parts per million
ppb	=	parts per billion (1 part in a thousand million)
mole	=	A mass equivalent to the relative molecular mass (or molecular weight) in grams. It may be expressed as mmole, nmole etc as above
mU		milliUnits, of enzyme activity, expressed in terms of the turnover of the appropriate substrate of the enzyme
mV		millivolts

Appendix 2
Questions posed to the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment by the Official Group on OPs

From: **Official Group on OPs. *Report to Ministers*, London: MAFF Publications, 1998.**

1. What evidence is there that it is possible to develop long-term symptoms from recognised acute organophosphate poisoning?

2. What evidence is there that an unrecognised acute poisoning incident could cause long-term effects?

3. What evidence is there that people can develop chronic symptoms from low-level exposure to organophosphates?

4. Does the picture become clearer if the studies to which the IEH report refers are divided up between those which purport to show objective or subjective end points?

5. Does the committee agree that a number of the studies discussed in the report had methodological flaws? If so, what were the flaws and how serious were they?

6. Is it possible, nonetheless, to deduce anything from these "flawed" studies?

7. Does the committee agree with the evaluation of the five particular studies suggested by Dr Ray as being the most reliable?

8. Is it possible that the chronic symptoms and signs from low-level exposure could be different from delayed symptoms from acute exposure?

9. Have the epidemiological studies that have been carried out on the effects of low dose exposure to organophosphates been of the right design and of sufficient statistical power to detect a biologically significant effect?

10. Is OP induced delayed polyneuropathy (OPIDP) relevant to the controversy about organophosphate sheep dips and does this aspect of the problem need further investigation?

11. What evidence is there of variable individual susceptibility to the extent that some people are more likely to suffer ill effects from OP exposure than others and could this account for long-term effects?

Organophosphates

12. Are there indications of possible mechanisms other than the inhibition of acetylcholinesterase at work?

13. Is it the committee's view that the animal models being used to investigate mechanisms of effects of OPs are the right ones to predict how OPs will affect humans?

14. What recommendations would the committee make for further research?

15. Is it the committee's view that those epidemiological studies, identified to be sufficiently well-conducted, could be used as the basis for a meta-analysis?

16. How do the symptoms experienced by those who attribute illness to OPs relate to effects observed in the epidemiological studies?

Appendix 3
Answers to questions posed by the Official Group on OPs

The original questions of the Official Group on OPs have been rephrased and grouped into subject areas. A cross reference to the original question number given in Appendix 2 is supplied.

Epidemiology

Question 1 (see original question 5)

What does the Committee consider are the important strengths and limitations of the epidemiological data concerning the risks of chronic health effects from low-level exposure to OP pesticides?

Strengths

A major strength of epidemiological research is its direct focus on human health, thus avoiding the uncertainties in extrapolating from animal data, or from *in vitro* laboratory studies. It also allows the investigation of psychological outcomes that cannot satisfactorily be studied in animals.

Weaknesses

All epidemiological studies are subject to weaknesses in their design or execution which limit their interpretation. In some cases these are unavoidable consequences of the practical and ethical constraints associated with research on humans. However, their impact can be reduced by good design. The main limitation of studies concerning the health risks from low-level exposure to OPs, include:

i. *Inappropriate study design.* Several studies have compared the findings from clinical and neuropsychological investigations in exposed subjects who have neurological symptoms with the findings in unexposed, asymptomatic controls. Where differences are found in such studies they cannot necessarily be attributed to exposure since they may simply reflect associations with symptoms irrespective of exposure.

ii. *Unrepresentative study samples.* Many epidemiological studies of the effects of OPs have low response rates and the possibility that those included were unrepresentative of the intended study population cannot be ruled out. In addition, many studies have only been concerned with current workers,

ignoring those who have left or retired from their jobs. This reduces their power to detect effects with a long latent period or any that were so severe that they would cause an individual to leave the workforce.

iii. *Crude or inaccurate measures of exposure to OPs.* When exposure is assessed from memory there is the potential for differential recall between those experiencing symptoms and those not experiencing symptoms thus creating 'recall bias'. In addition some studies have used crude indices of exposure and classified people as being exposed merely because they have been exposed to pesticides in general, because they were agricultural workers, or because they lived in agricultural areas. It is then difficult to attribute any findings specifically to an effect of OPs.

iv. *Biased assessment of health outcomes.* Many of the outcome measures have been subjective (e.g. whether or not symptoms have been reported) or semi-subjective (e.g. speed at completing an intellectual task) and a subject's knowledge of exposure may have biased his response or performance. Bias is also possible if classification of health outcomes depends on subjective assessment by a member of the research team who is aware of the subject's exposure status.

v. *Confounding.* Confounding is the process by which a non-causal association between two factors is produced by a third factor known as a confounder. To be a confounder a factor must be associated both with exposure to the suspect causal agent and also with the condition or disease under investigation. If a potential confounder is measured accurately, its effects can be taken into account, but this is not always possible. For example, potential confounders for neuropsychological testing include educational attainment, pre-exposure IQ, age, alcohol consumption and time of day of testing. In many studies information has not been available on all of these factors and so they could not be taken into account.

vi. *Size.* Most of the studies under consideration have been with exposed populations of 50-100 individuals and thus would not reliably detect moderately sized quantitative effects occurring only in a small subset (e.g. 5%) of the exposed population. Even if it produces a negative result a small study cannot give adequate reassurance that effects are not occurring in a small subset of the population.

vii. *Chance. The results of a study may be misleading simply by chance.* The potential for chance variation will depend on the size of the study (larger samples are less likely to be unrepresentative by chance) and can be quantitified statistically by confidence intervals and significance tests. However, even results that are highly significant statistically may sometimes be attributable to chance. When assessing the possible contribution of chance it is important to take into account biological plausibility and consistency with other observations. If results are inconsistent with a strong body of evidence

from other studies it is quite likely that they are either chance findings, or else due to bias or confounding.

In view of the above limitations no single epidemiological study can provide conclusive information in isolation. However, when all the most relevant studies are viewed together it is possible to draw useful conclusions.

Question 2 (see original Question 6)

Given these strengths and limitations what conclusions can be drawn from the epidemiology data regarding any risk of chronic health effects from low-level exposure to OPs?

The answer to this question is divided into whether effects are a long-term consequence of acute poisoning or of prolonged low-level exposure.

Long-term sequelae of acute poisoning

Neuropsychological outcomes

The balance of evidence supports the view that neuropsychological abnormalities can occur as a long-term complication of acute OP poisoning, particularly if the poisoning is severe. Such abnormalities have been most evident in neuropsychological tests involving sustained attention and speeded flexible cognitive processing ("mental agility"). In contrast, current evidence suggests that long-term memory is not affected after acute poisoning.

Peripheral neuropathy

Peripheral neuropathy, as one feature of OP-induced delayed polyneuropathy, is a well-established complication of poisoning by OPs that inhibit the enzyme NTE. The neuropathy is predominantly motor but possibly also sensory. Compounds that produce more than 70% inhibition of NTE give positive results in the hen test. Compounds evaluated as giving a positive response in the hen test are not used in the UK and have not been approved or licensed by regulatory agencies (i.e. the Veterinary Medicines Directorate or the Pesticides Safety Directorate).

The balance of evidence indicates that acute poisoning by other OPs, which do not inhibit NTE, can also lead to persistent peripheral neuropathy detectable by neurophysiological tests. If this occurs, most cases are not at a level that would give rise to symptoms.

Psychiatric illness

The limited evidence available does not allow any firm conclusions to be drawn regarding the risk of developing psychiatric illness in the long term as a consequence of acute poisoning by OPs.

Organophosphates

Prolonged low-level exposure

In comparison with the positive neurological and neuropsychological findings following recognised poisoning incidents, the evidence relating to chronic low-level exposure to OPs, insufficient to cause overt acute toxicity, is less convincing.

Neuropsychological outcomes

Although some studies suggest impairment in the same tests that are affected after acute poisoning, others do not. The balance of evidence does not support the existence of clinically significant effects on performance in neuropsychological tests from low-level exposures to OPs. If such effects do occur, they must either be relatively uncommon or so small that they are not consistently detectable by standard methods of testing.

Peripheral neuropathy

The balance of evidence indicates that low-level exposure to OPs does not cause peripheral neuropathy. If effects on peripheral nerve function sufficient to cause severe disability do occur, they must be rare.

Psychiatric illness

The available data indicate that exposure to OP sheep dips is not a major factor in the excess mortality from suicide among British farmers. However, in general, the evidence relating psychiatric illness to OPs is insufficient to allow useful conclusions.

Question 3 (see original question 7)

Does the Committee agree with the evaluation of the six particular studies suggested in the IEH Report as being the most reliable? (These were Stephens *et al.,* 1995, Maizlish *et al.,* 1987, Richter *et al.,* 1992, Fiedler *et al.,* 1997, Daniell *et al.,* 1992 and Ames *et al.,* 1995). If not, which studies do the Committee consider most reliable and why?

The Working Group identified 28 epidemiological studies (including the recent Institute of Occupational Medicine study) as being the most informative in relation to the question it was asked to address. Several of these have been published since the IEH Report was completed.

One of the six studies identified by the IEH as being of particular importance (Richter *et al.,* 1991) was not included in the 28 studies referred to above. It was excluded because it gave only a general summary of a programme of research that was mostly related to short-term toxic effects and it did not give a full description of any individual studies.

Question 4 (see original question 9)

Have the epidemiological studies that have been carried out on the effects of low dose exposure to organophosphates been of the right design and sufficiently large to have the statistical power to detect a biologically significant effect?

As would be expected there are limitations to all the available epidemiological studies. The specific limitations of those studies that the Working Group considered most informative are discussed in the critiques given in Appendices 4 and 5. These studies were generally not large enough to detect quantitative effects that occur only in a small proportion of the exposed population. Nor would they detect effects occurring in individuals who have left the work force because of ill health. They might also miss a small elevation of risk for relatively common disorders.

Question 5 (see original question 15)

The Working Group concluded at an early stage in its inquiry that the epidemiological studies identified could not be used as the basis for a formal meta-analysis. Nevertheless is it possible to reach an overall view on what evidence there is from these studies that people can develop chronic symptoms from low-level exposure to OPs?

Meta-analysis is a technique for pooling the results of two or more studies that addressed the same question. It is not appropriate here because the end-points measured in the published studies have varied considerably.

The Working Group's conclusions regarding the evidence that low-level exposure to OPs can cause chronic health effects are given in the answer to question 2.

Question 6 (a new question)

Can any conclusions be drawn from adverse reaction schemes, or from the information submitted to the Working Group relating to exposed persons?

The data available from HSE's Pesticide Incidence Appraisal Panel (PIAP) and VMD's Suspected Adverse Reaction Surveillance Scheme (SARSS) for veterinary medicines were of little value to the Working Group. These data mainly identify cases of acute rather than chronic effects and cannot be expected to detect non-specific effects occurring some considerable time after exposure. Although a number of symptoms had been reported following exposure to OPs these were mainly mild and, in the case of the PIAP data, transient. Also there was a lack of laboratory data to back up the symptoms reported. The information submitted to the Working Group by individual members of the public and patient groups indicated a high level of concern about potential long-term toxic effects of OPs. However, it was not possible to establish from individual case histories whether or not OPs can cause chronic illness.

Question 7 (see original question 4)

Does the picture become clearer if the studies to which the IEH Report refers are divided up between those which purport to show objective end-points (i.e. clinical signs or laboratory test data) or subjective end-points (i.e. symptoms)? Which are considered the most sensitive end-points (for OPs) and is there a pattern?

This question has now been overtaken by events as there are now a much larger number of studies than at the time of the IEH review. This question did not inform the Working Group's discussion and the key points are addressed in the answers to the following two questions. It was thus felt that no answer was necessary.

Question 8 (see original question 16)

How do the symptoms experienced by those who attribute illness to OPs, or which have been reported (in excess) following exposure to OPs, relate to the clinical signs observed or the symptoms reported in the epidemiological studies?

It was noted that there was inconsistency with regard to the results of the neuropsychological tests in the epidemiological studies and the reports from individual sufferers in that most of the epidemiological data did not suggest any effects on memory, nor was there any strong evidence for language impairment. Both of these complaints were common in the individuals who believed they had suffered OP poisoning. However, an effect on memory or language in a small sub-group of exposed people would not necessarily have been detectable in the epidemiology studies.

Question 9 (see original questions 3 and 8)

Is there evidence for the syndrome of chronic OP induced neuropsychiatric disorders (COPIND) produced by long-term low-level exposure to OPs (as defined by Jamal 1997)? If so how reliable is the evidence and what is the differential diagnosis for this condition?

COPIND (Jamal, 1997) is a possible sequel of both acute OP toxicity and chronic low-level OP exposure. The syndrome includes persistent impairment in a wide range of mental abilities (such as memory and attention) and in peripheral nerve function (sensory and motor neuropathy). The available evidence from epidemiological studies does not support the claim that this syndrome is caused by chronic low-level OP exposure. The studies reviewed provided weak evidence that low-level OP exposure results in cognitive impairment. In particular, no study, including those reporting positive findings on other measures, found an association between exposure and impairment of long-term memory. With regard to neuropathy, the findings from some studies are suggestive of an association between OP exposure and peripheral neuropathy, but other studies revealed little or no evidence of an effect. On balance, the Working Group concluded that the evidence did not support an effect of low-level exposure on peripheral nerve function and that if peripheral neuropathy does occur it must be rare.

The lack of evidence for the COPIND syndrome in the findings of studies examining the effects of low-level exposure to OPs may indicate that the syndrome is not a sequel of such exposure. On the other hand, the negative findings may merely reflect two important limitations of the majority of these studies: their focus on exposed individuals who belong to the workforce (thus excluding those who may have ceased work because of illness), and their unsuitability for detecting effects manifest in only a small sub-group of exposed individuals.

Consequences of different patterns of exposure

Question 10 (see original question 1)

What evidence is there that it is possible to develop long-term effects from well-recognised acute organophosphate poisoning?

There is good evidence from the epidemiological studies reviewed by the Working Group for the development of long-term neuropsychological effects and a predominantly motor neuropathy following episodes of acute poisoning. There was some evidence that this was dose-related. The strongest evidence related to persistent impairment in neuropsychological tests involving sustained attention and speeded processing but not memory.

It is recognised that high exposure to certain OPs may induce OP-induced delayed neuropathy (OPIDPN) and that this is closely associated with the ability of the OP to inhibit and age the enzyme neuropathy target esterase (NTE). OPs that are allowed for use as pesticides or veterinary medicines are investigated for this property as a crucial component of the approval process. However, there is the possibility that poisoning by other OPs may produce neuropathy, possibly sensory, through some other unknown mechanism.

Question 11 (see original question 2)

What evidence is there that an unrecognised acute or subacute poisoning incident, not causing frank intoxication, could cause long-term effects? Unrecognised incidents may be considered as:

- **unrecognised by regulatory authorities,**
- **unrecognised by the exposed person's doctor and not reported to authorities,**
- **unrecognised by the exposed person as meriting seeking medical advice.**

This has been answered in the comprehensive reply given to question 2.

Organophosphates **Mechanisms**

Question 12 (see original question 12)

Are there indications of possible mechanisms at work other than the inhibition of acetylcholinesterase? If so what are they?

There are a number of other putative mechanisms that may result in longer term toxic effects following exposure to OPs which are not directly related to inhibition of acetylcholinesterase. These are briefly listed below: they are considered in more detail in paragraphs 5.14 – 5.23 in the main text. In outline they are as follows:

- Phosphorylation of proteases, esterases or proteins involved in cell signalling
- Interaction with cytoskeletal proteins
- Excessive calcium influx in cells at nerve endings
- Prolonged receptor stimulation at nerve endings leading to muscle fasciculation and necrosis
- Hypoxic brain damage
- Psychological stress from an acute episode producing post-traumatic stress disorder

In two cases, namely inhibition of proteases and esterases and reaction with cytoskeletal proteins, the effects could be entirely independent of inhibition of acetylcholinesterase.

Question 13 (see original question 10)

Is OP-induced delayed polyneuropathy (OPIDN) relevant to the concern about organophosphate sheep dips and does this aspect of the problem need further investigation?

OPIDN is a well-recognised delayed consequence of acute poisoning with certain OPs. It is not directly relevant to this investigation which concentrates on long-term effects following exposure to levels that do not produce acute toxicity. OPIDN invariably occurs after episodes of marked acute toxicity and is only significant with certain OPs evaluated as giving a positive response in the hen test. The balance of evidence indicates that low-level exposure to OPs does not cause peripheral neuropathy. If effects on peripheral nerve function sufficient to cause severe disability do occur, they must be rare.

The current regulatory requirements for investigating whether an OP can produce OPIDN are based on the modern hen test and provide adequate data in this regard.

Question 14 (see original question 11)

What evidence is there of variable individual susceptibility such that some people are more likely to suffer ill health from OP exposure than others, and could this contribute to the long-term effects?

Individual susceptibility to OPs represents a balance between the rates of uptake into the body and then the comparative rates of activation and detoxification. It is known that there is considerable individual variability (up to 10- to 15-fold) in the activity of key enzymes in the process e.g. cytochrome CYP3A4 responsible for oxidative desulphuration of parathion; paraoxonase, an esterase in blood responsible for the hydrolysis of paraoxon. Some variability in enzyme activity has been noted between different racial groups. Thus, it is probable that some individuals are more susceptible than others to the effects of OPs. Differences in target organ sensitivity could also give rise to differences in response but there is no evidence to suggest that OPs are unusual in this regard.

Question 15 (see original question 13)

Is it the Committee's view that the laboratory models being used to investigate mechanisms of effects of OPs are the right ones to predict how OPs will affect humans?

The animal models that are currently used to support regulatory submissions provide a reasonable model of likely effects in humans. There is always some uncertainty in extrapolating results to humans and for this reason uncertainty factors are built into the assessment. It is also pertinent to note that the models are updated and improved to take account of advances in knowledge. For example the OECD guidelines for the 90 day repeated dose toxicity study were updated in 1998 to enhance their capability of detecting neurotoxic effects.

However, it must be recognised that animal models have limitations and currently cannot provide information on all the adverse effects that might be seen in humans e.g. psychiatric and some other behavioural effects. There is research in progress aimed at developing animal models to investigate mood and motivation.

Both *in vitro* and *in vivo* methods are valuable in investigating mechanisms of action of chemicals. Such knowledge reduces the uncertainty when extrapolating results seen in animal studies to humans. Basic physiology models in animals have value because they provide an understanding of the basis of the biological effects of chemicals.

Question 16 (new question)

Could there be interactions between OPs and other compounds to which individuals are exposed?

Theoretically, it is possible for interactions to occur both at the toxicokinetic (interference in metabolism) and the toxicodynamic (interference in tissue response) level. Toxicokinetic

aspects would occur when there is absorption of two or more compounds that share the same metabolic pathways for activation or detoxication. An enzymic pathway important in the metabolism of OPs involves a cytochrome referred to as cytochrome P-450 3A (or CYP3A). This pathway is also important for a range of drugs and certain chemicals that occur as natural constituents in food. However, such effects are, in general, only important at relatively high exposure levels, since at low levels there is usually sufficient metabolic capacity to cope with the multiple exposures with efficient detoxication and elimination of all the compounds.

Toxicodynamic aspects are less well understood. An example is the enhancement of the severity of OPIDN by subsequent exposure to promoter substances, i.e. substances that are not neurotoxic themselves but which can enhance the neuropathy caused by another agent, e.g. phenylmethanesulphonyl fluoride. However, exposure to such promoters is rare and should not present a major clinical problem in practice.

The possibility of interaction of OPs with anaesthetics, which may be due to a toxicodynamic rather than a toxicokinetic effect, has been highlighted in the Report of the Royal Colleges of Physicians and Psychiatrists. The Working Group considered the feasibility of work to investigate this specific concern. Although anaesthetic deaths are routinely recorded it was considered unlikely that such records would contain any information on previous exposure to OPs. Furthermore, it was considered more likely that OPs would prolong recovery from anaesthesia rather than cause mortality, and this would be difficult to investigate.

The original question 14 of the Official Group on OPs (concerning research recommendations) is covered in Chapter 9 of the main body of the report.

Appendix 4
Summaries and critiques of the epidemiological literature

Introduction

This appendix summarises the epidemiological studies published in the scientific literature that were considered by the Working Group. These are divided into those considered the most relevant to the work of the group and those that were less relevant. A detailed summary is given for each of the studies in the former group, together with a critique. Literature references for the studies in the latter group are listed, together with a brief explanation why they were considered to be less relevant.

The classification of studies as being most relevant to the work of the group depended on several factors. These included the strengths and limitations, as outlined in the Working Group's answer to question 1 of the Official Group on OPs (see Appendix 3). In addition, a critical consideration was the relevance of the study specifically to the question of whether exposure to low doses of OPs can cause long-term adverse health effects. The reasons why other studies were not considered as relevant are given at the end of this appendix. The exclusion of a study from the list of those most relevant should not necessarily be taken as indicating that it was scientifically a poorer study, only that it was considered to be less relevant to the inquiry of the Working Group.

Epidemiological studies considered most relevant to the inquiry of the Working Group

Ames RG, Steenland K, Jenkins B, Chrislip D, Russo J. Chronic neurologic sequelae to cholinesterase inhibition among agricultural pesticide applicators. Arch Environ Health 1995; 50: 440-443.

Amr MM, Halim ZS, Moussa SS. Psychiatric disorders among Egyptian pesticide applicators and formulators. Environ Res 1997; 73: 193-199.

Cole DC, Carpio F, Julian J, Leon N, Carbotte R, De Almeida H. Neurobehavioral outcomes among farm and nonfarm rural Ecuadorians. Neurotoxicol Teratol 1997; 19: 277-286.

Cole DC, Carpio F, Julian J, Léon N. Assessment of peripheral nerve function in an Ecuadorian rural population exposed to pesticides. J Toxicol Environ Health, Part A 1998; 55: 77-91.

Organophosphates Daniell W, Barnhart S, Demers P, Costa LG, Eaton DL, Miller M, Rosenstock L. Neuropsychological performance among agricultural pesticide applicators. Environ Res 1992; 59: 217-228.

Davies DR, Ahmed GM, Freer T. Chronic organophosphate induced neuropsychiatric disorder (COPIND): results of two postal questionnaire surveys. J Nutr Environ Med 1999; 9: 123-134.

Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol Appl Pharmacol 1979; 47: 161-176.

Engel LS, Keifer MC, Checkoway H, Robinson LR, Vaughan TL. Neurophysiological function in farm workers exposed to organophosphate pesticides. Arch Environ Health 1998; 53: 7-14.

Fiedler N, Kipen H, Kelly-McNeil K, Fenske R. Long-term use of organophosphates and neuropsychological performance. Am J Ind Med 1997; 32: 487-496.

Gomes J, Lloyd O, Revitt MD, Basha M. Morbidity among farm workers in a desert country in relation to long-term exposure to pesticides. Scand J Work Environ Health 1998; 24: 213-219.

Hawton K, Simkin S, Malmberg A, Fagg J, Harriss L. *Suicide and stress in farmers*, London: The Stationery Office, 1998.

Jager KW, Roberts DV, Wilson A. Neuromuscular function in pesticide workers. Br J Ind Med 1970; 27: 273-278.

London L, Myers JE, Nell V, Taylor T, Thompson ML. An investigation into neurologic and neurobehavioral effects of long-term agrichemical use among deciduous fruit farm workers in the Western Cape, South Africa. Environ Res 1997; 73: 132-145.

London L, Nell V, Thompson M-L, Myers JE. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense, and tremor among South African farm workers. Scand J Work Environ Health 1998; 24: 18-29.

Maizlish N, Schenker M, Weisskopf C, Seiber J, Samuels S. A behavioral evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. Am J Ind Med 1987; 12: 153-172.

McConnell R, Keifer M, Rosenstock L. Elevated quantitative vibrotactile threshold among workers previously poisoned with methamidophos and other organophosphate pesticides. Am J Ind Med 1994; 25: 325-334.

Misra UK, Nag D, Khan WA, Ray PK. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. Arch Toxicol 1988; 61: 496-500.

Otto DA, Sollman S, Svendagaard D, Soffar A, Ahmed M. Neurobehavioral assessment of workers exposed to organophosphorus pesticides. In: *Advances in neurobehavioral toxicology: applications in environmental and occupational health*, edited by Johnson BL, Anger WK, Durao A, Xintaras C. Chelsea, Michigan: Lewis Publishers, 1990, p. 306-322.

Pickett W, King WD, Lees RE, Bienefeld M, Morrison HI, Brison RJ. Suicide mortality and pesticide use among Canadian farmers. Am J Ind Med 1998; 34: 364-372.

Reidy TJ, Bowler RM, Rauch SS, Pedroza GI. Pesticide exposure and neuropsychological impairment in migrant farm workers. Arch Clin Neuropsychol 1992; 7: 85-95.

Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K, The Pesticide Health Effects Study Group. Chronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 1991; 338: 223-227.

Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health 1988; 43: 38-45.

Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. Chronic neurological sequelae to organophosphate poisoning. Am J Publ Health 1994; 84: 731-736.

Stephens R, Spurgeon A, Calvert IA, Beach J, Levy LS, Berry H, Harrington JM. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. Lancet 1995; 345: 1135-1139.

Stephens R, Spurgeon A, Berry H. Organophosphates: the relationship between chronic and acute exposure effects. Neurotoxicol Teratol 1996; 18: 449-453.

Stokes L, Stark A, Marshall E, Narang A. Neurotoxicity among pesticide applicators exposed to organophosphates. Occup Environ Med 1995; 52: 648-653.

Stoller A, Krupinski J, Christophers AJ, Blanks GK. Organophosphorus insecticides and major mental illness. An epidemiological investigation. Lancet 1965; I: 1387-1388.

Epidemiological studies considered to be less relevant

Aden-Abdi Y, Villén T, Ericsson Ö, Gustafsson LL, Dahl-Puustinen M-L. Metrifonate in healthy volunteers: interrelationship between pharmacokinetic properties, cholinesterase inhibition and side-effects. Bull World Health Org 1990; 68: 731-736.

Beach JR, Spurgeon A, Stephens R, Heafield T, Calvert IA, Levy LS, Harrington JM. Abnormalities on neurological examination among sheep farmers exposed to organophosphorous pesticides. Occup Environ Med 1996; 53: 520-525.

Behan PO. Chronic fatigue syndrome as a delayed reaction to chronic low-dose organophosphate exposure. J Nutr Environ Med 1996; 6: 341-350.

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Drenth HJ, Ensberg IFG, Roberts DV, Wilson A. Neuromuscular function in agricultural workers using pesticides. Arch Environ Health 1972; 25: 395-398.

Durham WF, Wolfe HR, Quinby GE. Organophosphorus insecticides and mental alertness. Arch Environ Health 1965; 10: 55-66.

Fleming LE, Bean JA, Rudolph M, Hamilton R. Mortality in a cohort of licensed pesticide applicators in Florida. Occup Environ Med 1999; 56: 14-21.

Horowitz SH, Stark A, Marshall E, Mauer MP. A multi-modality assessment of peripheral nerve function in organophosphate-pesticide applicators. J Occup Med 1999; 41: 405-408.

Jusic A, Jurenic D, Milic S. Electromyographical neuromuscular synapse testing and neurological findings in workers exposed to organophosphorous pesticides. Arch Environ Health 1980; 35: 168-175.

Korsak RJ, Sato MM. Effects of chronic organophosphate pesticide exposure on the central nervous system. Clin Toxicol 1977; 11: 83-95.

Levin HS, Rodnitzky RL, Mick DL. Anxiety associated with exposure to organophosphate compounds. Arch Gen Psychiatry 1976; 33: 225-228.

London L, Myers JE. Use of a crop and job specific exposure matrix for retrospective assessment of long term exposure in studies of chronic neurotoxic effects of agrichemicals. Occup Environ Med 1998; 55: 194-201.

Metcalf DR, Holmes JH. EEG, psychological, and neurological alterations in humans with organophosphorus exposure. Ann N Y Acad Sci 1969; 160: 357-365.

Parrón T, Hernández AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. Hum Exp Toxicol 1996; 15: 957-963.

Parrón T, Hernández AF, Villanueva E. Increased risk of suicide with exposure to pesticides in an intensive agricultural area. A 12-year retrospective study. Forensic Sci Int 1996; 79: 53-63.

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Rayner MD, Popper JS, Carvalho EW, Hurov R. Hyporeflexia in workers chronically exposed to organophosphate insecticides. Res Comm Chem Pathol Pharmacol 1972; 4: 595-606.

Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, Marzouk J, Levy S, Barron S, Gruener N. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. Isr J Med Sci 1992; 28: 584-598.

Roberts DV. E.M.G. voltage and motor nerve conduction velocity in organophosphorus pesticide factory workers. Int Arch Occup Environ Health 1976; 36: 267-274.

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Stålberg E, Hilton-Brown P, Kolmodin-Hedman B, Holmstedt B, Augustinsson K-B. Effect of occupational exposure to organophosphorus insecticides on neuromuscular function. Scand J Work Environ Health 1978; 4: 255-261.

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Factual summaries and critiques of the most relevant studies

Ames RG, Steenland K, Jenkins B, Chrislip D, Russo J. Chronic neurologic sequelae to cholinesterase inhibition among agricultural pesticide applicators. Arch Environ Health 1995; 50: 440-443

This study was designed to investigate whether exposure to OPs sufficient to produce acetylcholinesterase inhibition but no evidence of frank toxicity is associated with chronic neurological sequelae.

Study population

The study population comprised a sub-set of a larger National Institute of Occupational Safety and Health (NIOSH) Californian study on the chronic effects of OPs in people with a history either of frank toxicity or asymptomatic depression of acetylcholinesterase. Subjects were identified from medical supervision records for the years 1985, 1988 and 1989 and were subject to medical and neurological examination in 1990. They were asked to bring a friend of similar age who was not currently exposed to pesticides and did not have a history of pesticide poisoning and these formed the control subjects.

For this study a subset of 45 male subjects was identified with a prior history of documented cholinesterase inhibition which had led to their removal from occupational exposure, but who had not shown any evidence of frank toxicity. The criteria for such removal were that erythrocyte acetylcholinesterase activities were 70% or less of baseline, or plasma cholinesterase activities were 60% or less of baseline.

The control group consisted of 90 subjects selected from the pool of controls identified as described above. Slightly fewer of the exposed group were current smokers and drinkers, and they were on average older (38.2 *versus* 29.5 years) and of slightly lower educational attainment than the controls (mean grade 9.9 versus 10.6).

Exposure to pesticides

The assumption was made that workers who had been removed from exposure because of a low cholinesterase activity (the criterion for inclusion in the exposed group) had been exposed to one or more cholinesterase-inhibiting pesticides. Work histories could not be used to identify the compounds involved and it was not possible to study individual pesticides.

Neurological tests

A wide range of tests were carried out on each exposed and control subject. These involved nerve conduction velocity and amplitude tests (sensory in the median, ulnar and sural nerves; motor in the median and peroneal nerves), vibration sensation (finger and toe) neuropsychological tests (tapping, hand-eye, simple reaction time, sustained attention, symbol digit, pattern memory and serial digit) and studies to assess mood and motor coordination (pursuit aiming, Santa Ana dexterity and postural sway).

Results

One significant difference was seen when the results of the exposed and the control subjects were compared. There was a statistically significant enhanced performance in the exposed subjects in one of the tests in the neuropsychological battery, the serial digit performance test.

Critique

Adequacy of control group

The unexposed controls were friends of the exposed subjects or of other subjects who had experienced an episode of symptomatic pesticide poisoning. They were substantially different in age being younger than the exposed subjects, and were more likely to be white, somewhat better educated and were current drinkers and/or smokers.

Representativeness of samples

The exposed subjects were identified from medical supervision records, but this process is not clearly described. For example, it is unclear whether other exposed subjects could have been missed as a result of less frequent or less "luckily" timed acetylcholinesterase measurements than in the subjects who were included. Presumably all subjects who met the criteria during the relevant three years were included in the sampling frame but this is not stated and the response rate is unclear.

Exposure assessment

Characterisation as "exposed" depended on detection of below-threshold cholinesterase activities during monitoring. This classification is likely to have been fairly reliable.

Health outcomes

No evidence was found that OP or carbamate exposure, as assessed by prior blood cholinesterase inhibition in the absence of frank toxicity, is associated with chronic or long-term neurological sequelae.

Control of potential confounders

Adjustment was carried out for various potential confounders, although it may not have been sufficient to nullify the differences between the exposed and non-exposed groups.

Interpretation

Inhibition of cholinesterase activity did not appear to predict later neurological effects in the absence of acute symptoms. The study sample was relatively small and there are some uncertainties about the representativeness of both the exposed and non-exposed groups. Nevertheless the absence of a demonstrable difference from controls except in a single test from the neuropsychological battery is noteworthy and suggests at most a low incidence of long-term sequelae following exposures sufficient to depress cholinesterase activities.

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Amr MM, Halim ZS, Moussa SS. Psychiatric disorders among Egyptian pesticide applicators and formulators. Environ Res 1997; 73: 193-199

This was a cross-sectional study of pesticide applicators and formulators to assess psychiatric morbidity.

Study population

The exposed subjects included 208 formulators from two Egyptian plants where a wide range of pesticide products (organochlorines, OPs, carbamates, synthetic pyrethroids) together with chemicals were manufactured. These were randomly selected with the criterion that they must have been directly exposed to these chemicals for at least 40 hours per week for 9 months or longer, during at least two consecutive years. Also studied were 172 randomly selected pesticide applicators who had been involved in the annual application of pesticides (carbamates, pyrethroids, OPs and organochlorines, singly or in combination) for at least two consecutive years at two large model farms belonging to the Egyptian Ministry of Agriculture.

The control group comprised 72 workers from an urban textile factory who were matched with the pesticide formulators, and 151 members of a rural community who were matched with the pesticide applicators. The control subjects where chosen from the same communities as the exposed and were matched for age, socioeconomic status and educational level. None had a prior history of direct exposure to pesticides either at work or in the community.

Exposure to pesticides

The exposed group could have been exposed to a wide range of pesticides (not limited to OPs). No data were available on exposure levels. It was, however, stated that safety measures were generally poorly applied and workers lacked proper knowledge and training in the safe use of chemicals.

Neurological investigation

Psychiatric morbidity was assessed using a standardised screening tool, the General Health Questionnaire (GHQ) which provides information on somatic symptoms, anxiety/insomnia social dysfunction and severe depression. Diagnoses and classification were made in accordance with the revised edition of the Diagnostic and Statistical Manual of Psychiatric Disorders (DSM-III-R; American Psychiatric Association, 1989).

All subjects were assessed in the field by two psychiatrists. One administered the GHQ and the other assigned the DSM-III-R diagnosis.

Results

Significantly higher frequencies of psychiatric disorders were found in the pesticide formulators. The prevalence of depressive neurosis was 19.2% (40 out of 208) in this group compared to 6.9% (5 out of 72) in their controls ($p<0.05$). The corresponding values for situational/reactive depression were 19.2% (40 out of 208) versus 16.7% (12 out of 72)

($p<0.05$). The total prevalence of psychiatric disorders in the exposed group was 50% (104 out of 208) versus 32% (23 out of 72) in the controls ($p<0.01$). Statistically significant increases were also noted in the proportion of exposed workers, compared to controls, with symptoms of irritability 33.6% (70 out of 208) versus 13.2% (10 out of 72, $p<0.01$) and erectile dysfunction 26.9% (56 out of 208) versus 4.2% (3 out of 72, $p<0.001$).

Similarly, significantly ($p<0.005$) higher frequences in total psychiatric disorders were seen in the pesticide applicators (53 out of 172, 30.7%) compared to their controls (26 out of 151, 17.2%). Data specifically for dysthymic disorder were also given, showing a significant ($p<0.05$) excess in the pesticide applicators (41 out of 172, 23.8%) compared to their controls (22 out of 151, 14.6%).

Critique

Adequacy of control group

The exposed and control groups were matched on four important variables (age, socioeconomic status, educational level, and community) and this probably resulted in reasonably similar groups. However, little demographic information is given with which to compare the two groups and some doubt must remain about their comparability. It is implied, although not explicitly stated, that all members of both groups were male. For the pesticide formulators controls were chosen from another urban factory but the jobs of the controls for the pesticide applicators are not described.

Representativeness of sample

No information is given about the response rates. All groups included only current workers, so those who had retired or changed jobs were not represented.

Exposure assessment

As the exposed group consisted of current workers who had been in their present jobs for at least two years it is to be assumed that they had experienced both chronic and recent exposure. No data are given as to whether any of them had previously experienced acute poisoning. No biological measures of exposure status were obtained. Data are presented for two pairs of subgroups with unusually long or short employment duration, but comparisons between the pairs are hard to interpret as no information is presented as to whether these subgroups were chosen before or after the results were available and no data are presented on those with intermediate employment durations. No other quantitative measures of exposure were considered. The authors comment that safety measures were generally poorly applied and workers lacked proper knowledge or training in safe handling of chemicals. Thus exposures are likely to have been relatively heavy. Exposures involved organochlorines, pyrethroids, carbamates and other chemicals as well as OPs and no attempt was made to distinguish between the different chemicals or between types of OP. In consequence the conclusions are difficult to interpret in the context of UK practice and exposure.

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Health outcomes

Although it is not mentioned specifically, it appears unlikely that the psychiatrists carrying out the assessments were "blinded" as to the exposure status of the subjects, thus it is hard to rule out the possibility of systematic bias between the scoring of the two groups. Both the pesticide formulators and the applicators were shown to have a significantly higher prevalence of psychiatric disorders than their controls. There were also some significant differences for individual disorders and symptoms, although there may have been selective reporting of positive results as the total number of disorders and symptoms that were evaluated is not stated and different endpoints are reported for the two groups. There is clear evidence that the pesticide applicators were assigned higher psychiatric morbidity scores than the controls as the difference between the two groups was highly significant for the proportion classified as 'cases', the total score, and all four individual dimensions. However, no GHQ results are presented for pesticide formulators, again leaving open the possibility of selective reporting of positive results.

Control of potential confounders

As the unexposed subjects were matched to the subjects exposed to pesticides by community, age, socioeconomic and educational levels, confounding is unlikely to have been a major problem.

Interpretation

This study presents some evidence that working with pesticides under the described conditions is associated with increased levels of symptomatic psychiatric disorders. Unfortunately, however, some items of interest have been omitted from the study report and without these it is difficult to know how much weight can be attached to the findings.

Cole DC, Carpio F, Julian J, Leon N, Carbotte R, De Almeida H. Neurobehavioral outcomes among farm and nonfarm rural Ecuadorians. Neurotoxicol Teratol 1997; 19: 277-286

This was a cross-sectional study of farm workers in Ecuador to investigate neuropsychological effects. See also the next summary of Cole *et al*. 1998.

Study population

The 'exposed' population (n = 144) was identified by a health team carrying out a census of all those who lived on potato farms in Montufar canton in the northern province of Ecuador and were capable of doing farm work. These were compared with 72 age-, sex- and educationally-matched controls obtained from the local non-farm population. The 'exposed' subjects were divided into three sub-groups, those not doing direct farm work (consumers, n=23; the main constituent of their diet being the local potatoes treated with pesticides), those primarily working in the field and considered to be a generally exposed group (n=28) and those primarily applying pesticides (n=123). It was noted that because of the flexible nature of the work assignments amongst these small producers, applicators would also have conducted other work in the fields and the generally exposed group would also have been present during application. This is presumably why some individuals were included in more than one group.

Exposure to pesticides

The farm workers used a wide range of pesticides (43 in all), but three accounted for over 80% of the pesticide usage. These were carbofuran (a carbamate), methamidophos (an OP) and mancozeb (a diothiocarbamate). Application was almost always by means of a back-pack. Interviews with 40% of the applicators revealed a number of practices which would have increased exposures. These included mixing pesticides with the hands and using a stick (36 out of 40), leaking backsprays (28 out of 40), no use of protective clothing other than rubber boots (38 out of 40), storage of pesticides in the farm house (19 out of 40) and potentially unsafe pesticide disposal (35 out of 40). Erythrocyte acetylcholinesterase measurements revealed slightly reduced activities in all the 'exposed' groups, namely consumers, the generally exposed group and the applicators (11 to 15% lower than the control group, $p<0.001$).

Neurological tests

Each individual was subjected to a battery of neuropsychological tests based on the WHO Neurobehavioural Core Battery Test. This comprised digit span, Benton visual retention, digit symbol, simple reaction time, Santa Ana pursuit aiming test and profile of mood states (POMS). Three further tests were added because of their known sensitivity to acute poisoning by methamidophos, namely digit vigilance, Trails A and B and block design. In addition several language tests from the Weschler Adult Intelligence Scale (WAIS-verbal) were incorporated: these language tests were viewed as control variables tapping capacities unlikely to be affected by neurotoxic compounds. In the data analysis the individual tests were grouped into five 'domains' namely attention (digit vigilance and digit span); visuo-spatial (block design and Benton visual retention); psychomotor (digit symbol and Trails A

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and B); motor (visual and auditory reaction time, Santa Ana in the dominant hand, pursuit aiming); and affective (POMS).

In addition, all participants completed a brief medical questionnaire concentrating on those diseases that could independently affect outcome: information on alcohol use was also obtained.

Results

The farm population had a number of general deficits as compared to the controls. Considering the five overall neuropsychological function domains, the consumers did significantly worse ($p<0.10$) in tests for spatial, psychomotor and motor function, the regression coefficients and standard errors being -0.384 ± 0.1556, -0.477 ± 0.1374 and -0.241 ± 0.0991 respectively. The generally exposed group did significantly worse ($p<0.10$) on spatial tests, the regression coefficient being -0.306 ± 0.1459. Finally the pesticide applicators did significantly worse ($p<0.10$) on the attention tests with a regression coefficient of -0.345 ± 0.0786.

Critique

Adequacy of control group

Selection of controls is a serious problem with this study, limiting the conclusions that can be drawn. Two separate groups could be considered as unexposed controls. First, the nonfarm control population was intended to be matched on formal education as well as on age and sex, but the occupations listed include professional, skilled worker and small business person, which implies a degree of literacy and sophistication that almost certainly did not apply to the farm dwellers. The result is that the observed farm/nonfarm differences cannot be confidently attributed to OP exposure, but neither can this possibility be ruled out. Secondly, within the farm-dwelling group, the "consumers" are said to have done no farm work, and thus should not have had occupational exposures to pesticides, although they could have experienced environmental and/or dietary exposure. In addition, however, there are other ways in which they may have been similar to the occupationally exposed group that were unrelated to pesticides, for example relating to nutritional status, literacy and general education, and other factors that tend to differentiate rural and urban populations in a developing society.

Representativeness of samples

The sampling frame and the response rate are not completely clear, especially for the nonfarm control group. The *ex post* exclusion of farm-dwelling subjects on the grounds of age (<16 or >65) or fewer than four years of education was suboptimal.

Exposure assessment

In view of the very large number of compounds used in the area studied, information about specific exposures was hard to obtain; however, the majority of active substances appear to have been OPs, dithiocarbamates and carbamates. The timing of exposures in relation to testing was potentially problematic; as the authors say, when exposure is

ongoing, acute, sub-acute and chronic effects are all superimposed. Thus, recent exposure is a possible explanation for the observed effects.

Health outcomes

Great care appears to have been taken with the survey instruments and training, leading to high-quality and appropriate assessments.

Control of potential confounders

Adjustment for age and educational level was carried out, but this is unlikely to have been sufficient to remove the effects of socioeconomic differences between the rural and urban groups, as is indicated by the higher language-based scores among the control group.

Interpretation

Farm residents tended to have lower neuropsychological scores than nonfarm controls, after adjustment for age and educational level, and this could be due to pesticide exposure, but equally could relate to other differences between the groups.

Organophosphates

Cole DC, Carpio F, Julian J, Léon N. Assessment of peripheral nerve function in an Ecuadorian rural population exposed to pesticides. J Toxicol Environ Health, Part A 1998; 55: 77-91

This was a cross-sectional study of farm workers in Ecuador to investigate effects on the peripheral nervous system based on the same subjects as Cole et al. 1997 (see above).

Study population

The 'exposed' population (n = 144) was identified by a health team carrying out a census of all those who lived on potato farms in Montufar canton in the northern province of Ecuador and were capable of doing farm work. These were compared with 72 age, sex and educationally matched controls obtained from the local non-farm population. The 'exposed' subjects were divided into three sub-groups, those not doing direct farm work (consumers n=23, the main constituent of their diet being the local potatoes treated with pesticides), those primarily working in the field and considered to be a generally exposed group (n=28) and those primarily applying pesticides (n=123). It was noted that because of the flexible nature of the work assignments amongst these small producers, applicators would also have conducted other work in the fields and the generally exposed group would also have been present during application. This is most probably why some individuals were included in more than one group.

Exposure to pesticides

The farm workers used a wide range of pesticides (43 in all) but three accounted for over 80% of the pesticide usage. These were carbofuran (a carbamate), methamidophos (an OP) and mancozeb (a diothiocarbamate). Application was almost always by means of a back-pack. Interviews with 40% of the applicators revealed a number of practices which would have increased exposures. These included mixing pesticides with the hands and using a stick (36 out of 40), leaking backsprays (28 out of 40), no use of protective clothing other than rubber boots (38 out of 40), storage of pesticides in the farm house (19 out of 40) and potentially unsafe pesticide disposal (35 out of 40). Erythrocyte acetylcholinesterase measurements revealed slightly reduced activities in all the 'exposed' groups, namely consumers, the generally exposed group and the applicators (11 to 15% lower than the control group, $p<0.001$).

Neurological tests

Peripheral nerve function

Each individual was subjected to a range of studies to investigate peripheral nerve function. Neurological tests comprised measurement of coordination and balance (index finger-nose test, heel-ankle test, Romberg test (standing with arms outstretched and closed eyes), and Mingazini test (maintaining lower leg upright whilst lying face down), and an appraisal of gait. Also assessed were deep tendon reflexes in the upper and lower limbs (radialis, biceps, knee and ankle) bilaterally, muscle power at 16 sites using the MRC (1943) scale, and vibration sensitivity in the big toe. Individuals were asked to complete a questionnaire involving yes/no responses relating to symptoms in the legs ('gone to sleep', paraesthesiae, progressive weakness and flaccidity).

Results

Peripheral neuropathy outcomes

Comparison of the pesticide applicators with the controls using polytomous logistic regression analysis revealed a higher frequency of several parameters relating to peripheral neuropathy. There was an excess of current peripheral neuropathy symptoms (Odds Ratio (OR) 3.1, $p<0.001$), signs of poor coordination (OR 4.3, $p<0.001$), abnormal deep tendon reflexes (OR 2.9, $p<0.001$) and reduced muscle power (OR 2.1, $p=0.12$). Deep sensation was not significantly different. In addition, analysis of mean big toe vibration threshold scores by multiple linear regression indicated that these were higher in pesticide applicators ($p=0.087$), particularly in those reporting previous pesticide poisoning ($p=0.008$).

Critique

Adequacy of control group

Selection of controls is a serious problem with this study, limiting the conclusions that can be drawn. Two separate groups could be considered as unexposed controls. Firstly, the nonfarm control population was intended to be matched on formal education as well as on age and sex, but the occupations listed include professional, skilled worker and small business person, which implies a degree of literacy that almost certainly did not apply to the farm dwellers. Secondly, within the farm-dwelling group, the "consumers" are said to have done no farm work, and thus should not have had occupational exposures to pesticides, although they could have experienced environmental and/or dietary exposure. In addition, however, there are other ways in which they may have been similar to the occupationally exposed group that are unrelated to pesticides, for example relating to their nutritional status, literacy and general education, and other factors that tend to differentiate rural and urban populations in a developing society. The result is that the observed farm/nonfarm differences cannot be confidently attributed to exposure to OPs, but neither can this possibility be ruled out.

Representativeness of samples

The sampling frame and the response rate are not completely clear, especially for the nonfarm control group. The *ex post* exclusion of farm-dwelling subjects on the grounds of age (<16 or >65) or fewer than four years of education was suboptimal.

Exposure assessment

In view of the very large number of compounds used in the area studied, information about specific exposures was hard to obtain; however, the majority of active substances appear to have been OPs, dithiocarbamates and carbamates. The timing of exposures in relation to testing was potentially problematic. As the authors state, when exposure is continual, acute, sub-acute and chronic effects are all superimposed. Thus, recent exposure is a possible explanation for the observed effects.

Organophosphates

Health outcomes

Great care seems to have been taken with the survey instruments and training, leading to high-quality and appropriate assessments.

Control of potential confounders

Adjustment for age and educational level was carried out, but this is unlikely to have been sufficient to remove the effects of socioeconomic differences between the rural and urban groups, as is indicated by the higher language-based scores among the control group.

Interpretation

The cluster of adverse findings among the most highly exposed subjects strongly suggests that the peripheral nervous system may be adversely affected by the pesticides used. This was apparently not a result of previous episodes of acute poisoning, but it is unclear whether the effect resulted from recent exposure or from exposure of longer duration.

Daniell W, Barnhart S, Demers P, Costa LG, Eaton DL, Miller M, Rosenstock L. Neuropsychological performance among agricultural pesticide applicators. Environ Res 1992; 59: 217-228

This was a prospective longitudinal study of neuropsychological performance in apple orchard pesticide applicators over one growing season compared to beef slaughterhouse workers.

Study population

The exposed population was obtained from agricultural pesticide workers in Yakima Valley, Washington State, USA. They were selected initially by identifying growers from a trade association who were willing to participate. Subsequently individual workers were approached directly for enrolment on a voluntary basis. A total of 57 applicators agreed to participate from 16 orchards; the total eligible population was not reported. Eight (14%) of the initial study group were not able to participate in the post-season follow-up six months later and the reasons for this were not given.

The control group comprised 51 beef slaughterhouse workers recruited from one site. Again there was some loss (11, 22%) at the post-season follow-up. Thus the final study sample consisted of 49 exposed and 40 control individuals.

There were significant differences between the exposed and control populations with respect to language and educational attainment. The exposed cohort had a much greater proportion of individuals with a preference for use of Spanish in the tests (22 out of 49, 45%) compared to the controls (5 out of 40, 12%). After stratification by language preference there was no significant difference in the background characteristics of the exposed and control groups apart from fewer years of education in the exposed Spanish-speaking subjects (mean 5.0 years *versus* 7.8 years).

Exposure to pesticides

The pesticide applicators were exposed mainly to azinphos-methyl. More than half (57%) reported spending 10 or more days directly involved in pesticide spraying activities during the study season, 35% spent less than 10 days (but some involvement), and 8% had only proximate exposure without direct involvement in spraying. There were no details of exposure measurements.

Neuropsychological evaluation

Each individual in the study was subjected to a pre-season (January or February) and a post season (September or October) evaluation with a period of at least four weeks between the last pesticide application and the evaluation.

Evaluations consisted of a questionnaire relating to a history of pesticide use, demographic background and medical history. Information was also requested on a number of factors in the preceding 24 hours which could influence test performance. Blood samples were taken for acetylcholinesterase activity measurements. The individuals then underwent a battery of

Organophosphates neuropsychological tests utilising the English or Spanish versions of the Neurobehavioural Evaluation System (NES). The test battery included 5 NES subtests namely finger tapping, hand-eye coordination, continuous performance (a choice or complex reaction test conducted over five minutes), symbol digit test (modification of the Weschler Adult Intelligence Scale, WAIS, digit symbol subtest), and a pattern memory test. It was decided *a priori* not to include the NES vocabulary subtest because of the anticipated language and educational differences within the study sample. It was recognised that such differences would invalidate the use of these tests as potential indicators of either pre-exposure intelligence or exposure effects.

Results

With regard to pre- and post-seasonal analysis of the exposed group it was noted that pre-seasonal baseline performance was a significant predictor of post-seasonal test performance. After controlling for language preference there was no statistically significant impairment in the performance of the exposed group in any of the neuropsychological tests when compared with the controls. The only statistically significant deterioration over the spraying season related to one test in the subgroup of exposed subjects with Spanish language preference and only when adjusted for pre-season performance. This was in the symbol digit substitution test.

Critique

Adequacy of control group

The beef slaughterhouse workers were much less likely than the applicators to prefer the Spanish-language edition of the test battery and to have less than six years of education, but were quite well matched in other respects. However, more than a quarter of them reported having worked with pesticides at some time, and over half had picked or trimmed crops. This would have tended to obscure any long-term effects of pesticide exposure.

Representativeness of samples

The sampling frame for applicators was unknown, and the sample should be considered as a volunteer ("convenience") sample. The loss to follow-up in the post-season assessment is a further limitation. The study size was small, and differences between the exposed and control groups in language preference further reduced the statistical power of the study.

Exposure assessment

Pesticide exposure assessment was by self-administered questionnaire, and for the post-season evaluation was retrospective for the recent spraying season. Exposures were considered to be low, and not all the applicators reported direct involvement in spraying. Little information was available on specific pesticides, although azinphos-methyl is mentioned as the predominant one. There is no mention of blinding in the assessment of health outcomes.

Health outcomes

The study found no evidence of clinically significant adverse changes following pesticide exposure, apart from an isolated finding of a decrement in the symbol digit substitution test.

Control of potential confounders

This appears to have been satisfactory.

Interpretation

In principle, the controlled before/after design is a good one. The problems with this study are the small size and heterogeneity of its sample, and possible prior pesticide exposure of controls. No impairments were demonstrated at these low exposure levels.

Organophosphates

Davies DR, Ahmed GM, Freer T. Chronic organophosphate induced neuropsychiatric disorder (COPIND): results of two postal questionnaire surveys. J Nutr Environ Med 1999; 9: 123-134

This investigation consisted of two postal surveys, each using a postal questionnaire, of neuropsychiatric symptoms in individuals exposed to OPs.

Study population

The first survey focused on 400 farmers selected at random from the Yellow Pages for Cornwall and West Devon (choosing every fifth entry from the individual names listed until 400 subjects were identified). A total of 179 (45%) responded but four were excluded leaving a study sample of 175 individuals. Of these 45 (26%) reported no exposure to OPs.

The second survey comprised 240 individuals identified from the OP Information Network database as having registered concerns about ill health possibly related to OP exposure. A total of 215 (90%) responded but four were excluded for various reasons, leaving a study sample of 211 individuals. Of these 179 (85%) were considered as being exposed to OPs via sheep-dips (many had also been exposed in other ways). Thirty-two individuals had had no involvement with sheep dipping but had been exposed to OPs in other ways.

Exposure to pesticides

Exposure was assessed from the answers to the questionnaire.

In the first survey this was based on the answer to a simple question as to whether the individual had used or been exposed to OPs.

In the second survey more detailed information was requested on the type of exposure, whether it involved concentrate, and its duration.

The information was not given on the identity of the specific OP to which individuals were exposed.

Assessment of symptoms

In the first study, simple yes/no questions were asked relating to the 10 key symptoms of COPIND. These were exacerbation of dipper's flu, personality change, suicidal thoughts, cognitive impairment, language disorder, alcohol intolerance, heightened sense of smell, handwriting deterioration, OP sensitivity and decreased exercise tolerance.

A more detailed questionnaire was used in the second survey relating to symptoms of COPIND, and other symptoms, again in a yes/no format.

Results

In the first survey a comparison was made between individuals exposed and not exposed to OPs. The numbers of individuals with 0, 1-2, 3-4, or 5-8 symptoms in the exposed group were 53 (41%), 24 (19%), 21 (16%) and 32 (25%) respectively. The corresponding values in the non-exposed group were 39 (87%), 4 (9%), 2 (4%) and nil. There was a highly statistically significant ($p<0.0001$) excess of symptoms in the group exposed to OPs.

In the second survey a very similar pattern of symptoms was noted in those exposed to OPs through the use of sheep dips, and those exposed to OPs from other sources. Furthermore this was similar to that seen in those exposed to OPs in the first survey.

Critique

Adequacy of control group

In the first survey, the control group consisted of those who reported no exposure to OPs in the past 10 years. This characterisation was thus based on self-reports, with no detail on the time relationship between exposure and the onset of symptoms. The group consisted of only 45 respondents.

Representativeness of samples

With a response rate of 44 percent to the first survey, it is unclear how similar the respondents were to the non-respondents. The authors argue plausibly that response bias is likely to have been balanced by other factors, but this cannot be clearly established. The second study had a high response rate, but the sampling frame (the OPIN database) was composed of people who report both OP exposure and neuropsychiatric symptoms and therefore cannot be considered representative of people with either attribute alone.

Exposure assessment

Exposure status was based solely on self reports.

Health outcomes

The focus of the paper is on the clustering of the ten component symptoms of the proposed syndrome of COPIND, and this is its main importance. The proportions reporting the various items are indeed similar in the three groups (exposed in the first study; individuals from the OPIN database exposed to OP sheep dips, and in other ways in the second). However, more convincing evidence would have been obtained if the questionnaire had also contained other ("dummy") symptoms, not thought to be part of COPIND, which would make it possible to establish whether there is a preferential tendency for these particular items to cluster together.

Control of potential confounders

Confounding did not appear to have been considered. This would be important if the principal aim of the paper were to investigate the possible causal association of the syndrome and OP exposure, rather than to establish the component symptoms of the syndrome.

Interpretation

The paper provides preliminary evidence of the components of the proposed syndrome, but on its own cannot be said to establish that the syndrome exists as a distinct entity. It also provides only suggestive evidence of a link with OP exposure. .

Organophosphates

Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol Appl Pharmacol 1979; 47: 161-176

An investigation of the electroencephalogram (awake and asleep) of industrial workers who had accidentally been exposed to the nerve gas sarin at levels producing acute toxic effects at least one year before the study.

Study population

The 'exposed' population consisted of 77 workers who had a history of accidental poisoning by sarin. This was defined by the following criteria; a verified history of discrete exposure resulting in clinical symptoms and signs consistent with exposure; reduction of erythrocyte acetylcholinesterase to an activity at least 25% below the individual's pre-exposure activity. None had been exposed during the year preceding the study. Forty one of the workers who had experienced three or more accidental exposures in a six year period formed a higher exposure sub-group.

The control group consisted of 38 workers from the same plant who had not experienced accidental exposure to sarin, and had never complained of symptoms of OP toxicity or shown significant fluctuation in acetylcholinesterase activities during semi-annual or spot checks. Members of this control group were chosen so that their age distribution and socioeconomic background matched that of the exposed group. All subjects were male. The mean age of the control group was 42 years compared to 46 in the exposed group.

Exposure

This was as described above.

EEG evaluation

Three separate investigations were undertaken.

i. ***Spectral analysis of tape recorded EEG***

Subjects were studied in a standard EEG laboratory under the following conditions; eyes open (2 min during which subjects were frequently alerted by auditory stimuli); eyes closed (2 min); drowsy (15 minutes in dark with extraneous sounds masked with white noise); hyperventilation (5 minutes); recovery from hyperventilation (3 min).

ii. ***Visual inspection of routine clinical EEG***

Routine clinical EEG was performed using a Grass model 78 EEG with complete 24 electrode montage.

iii. **Visual inspection of an all night sleep EEG**

An all night sleep EEG was recorded for each subject during his usual sleep period.

Results

Workers with a history of exposure to sarin had waking and sleeping EEGs that differed significantly from workers without exposure in both univariate and multivariate discriminant analysis (p=0.001 for the whole exposed group and p=0.0001 for the 'high' exposure sub-group). Statistically significant differences included increased beta activity, increased delta and theta slowing, decreased alpha activity and increased amount of rapid eye movement sleep. These differences were clear at the group level. It was not, however, possible to diagnose subjects on an individual basis by expert visual inspection of their EEG.

The same authors have also reported significant EEG changes (persistent increases in relative amount of high frequency beta activity) in rhesus monkeys following lower exposure to sarin not associated with major signs of toxicity (1 µg/kg once a week over 10 weeks). This study was reported later together with a repetition of the results for the human studies noted above [Burchfiel JL, Duffy FH. Organophosphate neurotoxicity: chronic effects of sarin on the electroencephalogram of monkey and man. Neurobehavioral Toxicology and Teratology 1982; 4: 767-778].

Critique

Adequacy of control group

The use of other workers in the same plant was appropriate, but their mean age was slightly lower than that of the exposed group. Also, although the salary levels are described as similar, an unspecified number of unexposed controls worked in different types of occupations, e.g. as janitors, clerks or guards, rather than as operatives with (non-OP) chemical exposures.

Representativeness of samples

The selection and recruitment of subjects is not clearly described, and no response rates are given. The control group were chosen so that their age and sex distribution and socioeconomic background matched the exposed group, but it is not clear that this involved stratified random sampling.

Exposure assessment

Exposed subjects had had one or more accidental acute exposures to sarin in the past, none being within the previous 12 months; it is unclear whether they could have been exposed to any other OPs. The definition of an exposure event was specified in relation to known sarin exposure, appropriate symptoms and signs, and reduction in erythrocyte acetylcholinesterase activity of at least 25 percent below baseline. No participant had a history of exposure to carbamates or chlorinated hydrocarbons.

Health outcomes

The proportion of EEGs that were judged "abnormal" was unusually high: 24 percent among unexposed controls and 29 percent in the exposed group (the difference not being statistically significant). The reason for this is unclear. The main findings relate to

differences in various features of the EEG record that were unrelated to the judgement of abnormality. Thus they may not have been clinically important.

Control of potential confounders

No mention is made of control for confounders, except that for the all-night-sleep EEG the few subjects younger than 30 years were excluded to render the populations more comparable.

Interpretation

The shortcomings of the study design are unlikely to explain the findings. In addition, a parallel experimental study using adult rhesus monkeys showed comparable findings [Burchfiel JL, Duffy FH. OP neurotoxicity. Chronic effects of sarin on the EEG of monkey and man. Neurobehavioural Toxicology and Teratology 1982; 4: 767-778]. In the latter paper, the authors also show that their findings in humans, using discriminant function analysis, are compatible with a bimodal distribution, one peak coinciding with that of the unexposed control group. This suggests a threshold for long-term effects or sub-group differences in susceptibility. The findings suggest that short-term accidental exposure to sarin can cause persistent EEG abnormalities.

Engel LS, Keifer MC, Checkoway H, Robinson LR, Vaughan TL. Neurophysiological function in farm workers exposed to organophosphate pesticides. Arch Environ Health 1998; 53: 7-14

This was a cross-sectional study to investigate neurophysiological function in apple thinners exposed to low levels of OPs (mainly azinphos-methyl) *via* residues on leaves (no direct exposure during pesticide application).

Study population

The exposed group was selected from people aged 16 to 45 years residing in the Wenatchee area of Central Washington State, USA. For inclusion in the study they had to have lived in the area for at least one year and worked as apple thinners for 80 hours or longer in the current season; furthermore they had to be available for follow-up testing in the spring following the thinning season. A total of 69 Hispanic farm workers were identified, of whom 68 agreed to participate: one was later excluded because he was over 45 years of age. None of the exposed group had any history of pesticide use during the current season. The control group was selected from various local factories and service industries. From the 76 selected who met the study selection criteria and who were matched by age, gender, ethnicity and educational level with the exposed subjects, 69 agreed to participate but one was excluded. The control group was also closely matched to the exposed group with regard to alcohol consumption. About half of the exposed and half of the control group had worked on farms in previous seasons.

Exposure to pesticides

The exposed group (apple thinners) were exposed primarily to azinphos-methyl and possibly to phosmet or methyl parathion *via* foliar OP residues. This 'indirect' exposure can be assumed to be low and their erythrocyte acetylcholinesterase activity was normal at the time of the neurophysiological examination.

Neurophysiological examination

Nerve conduction measurements were carried out in the upper and lower limbs. Sensory nerve conduction velocity was measured in the sural nerve and motor conduction velocity in the ulnar nerve. Neuromuscular junction function was measured by repetitive stimulation of the ulnar nerve at the wrist, at rest, after 30 seconds of maximal isometric exercise, and two minutes after exercise. Post-exercise facilitation and exhaustion were assessed from these data.

Results

No statistically significant neurophysiological differences were noted between the exposed and control groups. Each had similar sensory nerve latency and amplitude (sural), motor nerve conduction velocity (ulnar), and neuromuscular junction function (ulnar).

In an attempt to investigate dose-response relationships, the exposed workers were graded according to time spent in apple thinning during the season. There was no relationship between duration of exposure and the results of the electrophysiological studies.

Organophosphates **Critique**

Adequacy of control group

Matching was carried out for gender, ethnicity, five year age group and three year educational group. The non-exposed controls were a heterogeneous group, but the matching process probably ensured socio-demographic comparability. Many of the control group had engaged in agricultural work of some kind, in the present or previous seasons.

Representativeness of samples

The sampling frames were clear-cut, and the response rates were over 90 percent.

Exposure assessment

In addition to an entry criterion (80 or more hours of apple thinning during the current season, regarded as a highly exposed occupation), questionnaire-based information was also available. In addition, detailed information was available on a sub-sample.

Health outcomes

No differences in peripheral nerve conduction or neuromuscular function were detected.

Testing of the outcome variables was carried out by persons blind to exposure status.

Control of potential confounders

Multivariate analyses and a well-matched control group probably ensured that appreciable confounding did not occur in this study.

Interpretation

This study provides some evidence that peripheral neurophysiological function is not impaired at this level of exposure to OPs, which is likely to have been low.

Fiedler N, Kipen H, Kelly-McNeil K, Fenske R. Long-term use of organophosphates and neuropsychological performance. Am J Ind Med 1997; 32: 487-496

This was a study to assess neuropsychological function and psychiatric variables in tree fruit farmers who had been involved in long-term use of OPs with no history of acute poisoning.

Study population

The exposed group were tree fruit farmers who were also licensed pesticide applicators. They were identified from a postal survey of such farmers in two counties in New Jersey; 84 out of 216 of those approached (39%) agreed to participate but 27 were rejected for various reasons (age, other illnesses, lack of exposure to OPs or scheduling conflicts). Thus the exposed group comprised 57 farmers.

The control group was chosen from blueberry/cranberry farmers from the same two counties: this occupational group would be expected to have little or no exposure to pesticides. The response rate to the postal survey of this group was poor (34 out of 237, 14%) and 11 of those who agreed to participate were rejected for various reasons (age, inadequate exposure data, poor reading ability or scheduling difficulties). Thus there were only 23 'control' fruit farmers. These controls were therefore supplemented by a number of hardware store owners from the same area selected by the same postal procedures. However, here the response rate was even poorer (22 out of 285, 8%) two of whom were rejected (health and scheduling problems). The average age, years of education and pre-morbid intellectual ability (as measured by the Wide Range Achievement Test Reading (WRAT-R) score was the same as for the blueberry/cranberry growers and so both groups were combined to form one control population of 44 subjects.

The ages of the exposed and control groups were similar but the controls had on average a higher length of education (14.3 years *versus* 13.1 years) and WRAT-R score (99.9 *versus* 93.1).

Exposure to OPs

Data on exposure to OPs were limited. The exposed group was selected from an occupational group expected to be exposed to OPs. An attempt was made to grade exposure using a structured questionnaire about acreage, extent of pesticide use and protective clothing, but no details were given; it was stated that such data would be reported separately but the Working Group are not aware of any such later publication. None of the pesticide exposed group had any history of acute poisoning that resulted in their seeking medical advice, except for one individual with an eye splash that did not produce any symptoms of effects on the central nervous system. All erythrocyte acetylcholinesterase measurements were within the normal range.

Organophosphates

Neuropsychological and psychiatric studies

A wide range of neuropsychological tests were carried out to investigate the following functions; concentration (simple reaction time, computerised visual reaction time, choice reaction time over five minutes and Stroop colour word task): visuomotor skills (NES battery hand-eye coordination, grooved pegboard test) Trails A and Trails B and revised Wechsler Adult Intelligence Scale WAIS-R (digit symbols); verbal memory (WAIS-R digit span and Californian Verbal Learning Test); visual memory (Wechsler memory scale revised and continuous visual memory); verbal ability (information subset of WAIS-R); and expression and receptive language (animal naming test and revised token test).

Personality traits and possible psychiatric and neurological morbidity were assessed using the Minnesota Multiphasic Personality Inventory (MMPI-2).

Other medical information

Detailed medical histories were obtained and a physical examination was carried out on each subject concentrating upon neurological function. In addition, clinical chemistry and haematology with erythrocyte acetylcholinesterase determination were performed. No subject was excluded because of drug or alcohol abuse. One of the blueberry/cranberry workers was excluded because of visual defects.

Results

The sole significant difference in the neuropsyschological tests of the exposed as compared with the non-exposed group was a statistically significant slower response on the simple reaction time test. Mean values for the dominant hand were 293.6 msec *versus* 263.8 ± 8.5 msec in the controls (p=0.01). The effects were somewhat less marked when the comparison was between 'high' exposure and 'low' exposure groups.

With regard to the 10 clinical scales measured in the MMPI-2 test, the only significant difference was a lower value on the mean masculinity-femininity score in the exposed workers 42.3 versus 45.4 in the controls: (p=0.05). There was no evidence of any deficit with regard to emotional status or personality.

Critique

Adequacy of control group

The berry growers were probably well chosen as a control group in sociodemographic terms; it has to be taken on trust that they would have little or no exposure to pesticides. However, they were supplemented with an almost equal number of hardware store owners, whom one would expect to be substantially different from tree fruit farmers. The absence of statistically significant differences was an unsatisfactory basis for combining the two control groups in view of the small numbers in each group (23 and 21). It was found that the exposed and control groups differed significantly in years of education and in reading test scores.

Representativeness of samples

The response rates were very low (<30 percent in the exposed group, approximately 11 percent in the controls, if "scheduling conflicts" are judged to be a reason for non-response rather than for non-eligibility). They were essentially volunteer samples.

Exposure assessment

Some subjects were re-allocated between exposed and non-exposed groups following a retrospective assessment of lifetime exposure.

Health outcomes

Reaction time was the only one of many attributes that was found to be related to exposure status.

Control of potential confounders

Adjustment for reading test scores may not have completely removed the effect of confounding differences between the groups.

Interpretation

This is a small study of volunteer samples, providing weak evidence of an association of reaction time with exposure, together with weak evidence that the other measured attributes are not associated with exposure.

Organophosphates

Gomes J, Lloyd O, Revitt MD, Basha M. Morbidity among farm workers in a desert country in relation to long-term exposure to pesticides. Scand J Work Environ Health 1998; 24: 213-219

This study was designed to assess subclinical morbidity patterns among male farm workers in the United Arab Emirates exposed to OP pesticides.

Study population

Expatriate workers from developing countries, predominantly ethnic Asians, employed in farming, in industry and as domestic helpers in the United Arab Emirates were recruited for the study. The 'exposed' group consisted of established farm workers (n=226); who had worked for at least two years in their present jobs. These were compared with a 'control' group of referents matched for age and nationality (n=226) who had worked for at least two years in non-agricultural jobs with no occupational or domestic exposure to pesticides. In addition an unmatched group of farm workers (n=92), newly arrived in the country but having worked for at least two years in the farming industry in their own country, was also investigated. The response rate was 99%.

Exposure to pesticides

No details are given as to the type and extent of exposure to specific pesticides. However, determination of the activity of acetylcholinesterase in whole blood was used as an objective measure of exposure. Mean erythrocyte acetylcholinesterase activities were reduced by 16% in the exposed group compared to the controls ($p<0.01$) indicating some exposure to OPs. There was no reduction of erythrocyte acetylcholinesterase activity in the new farm workers.

Neurological tests

Neurological dysfunction, memory disorder and loss of neuromuscular coordination were assessed by the digit symbol test and the aiming test under controlled, comfortable conditions.

Other medical information

A questionnaire was administered to obtain information on demographic characteristics, health status, work and dietary habits. Blood pressure, heart rate, height and weight were measured and the body mass index was calculated. An occupational physician, "blind" to the subjects' jobs, sought information as to their past and present health status including symptoms that might be associated with organophosphate toxicity. The subjects' health status was assessed by an examination of their conjunctiva, their vision, for chest wheeze, and for the condition of the skin on their hands.

Results

There were no significant differences in body mass index, systolic and diastolic blood pressures or heart rate between the three groups. The scores of the established farm workers, in the aiming test and the digit symbol test were significantly lower than those for

the referents. Mean values obtained (± SD) were: aiming test 7.17 ± 12.05 *versus* 18.98 ± 18.55, p<0.01; digit symbol test 0.90 ± 3.83 *versus* 5.97 ± 11.70, p<0.01. Similar reductions were seen in the new farm workers (where there was no indication of exposure to OPs from erythrocyte acetylcholinesterase activities) but this group was not matched.

Regarding the morbidity profile, irritated conjunctiva, watery eyes, blurred vision, runny nose, wheeze and chest tightness in the last month were all reported significantly more frequently in the established farm workers. Similarly, a significantly greater number of established farm workers than of referents or new farm workers reported dizziness, headache, restlessness, sleeplessness, muscular pain, abdominal pain or weakness as occurring often or sometimes. This was statistically highly significant in all cases (p<0.001) with 85 to 100% of the 'control' group not reporting such symptoms whereas the prevalence rates for the exposed group were 47% to 77% depending on the symptom.

Critique

Adequacy of control group

In the design of their study, the authors made good use of the fact that attendance at an occupational health clinic was compulsory for expatriate workers. The study was limited to males, matched for age and nationality, and both established workers in other jobs and farm workers, both new to, and established in, the United Arab Emirates were considered. Thus the study allowed differentiation of general differences between those employed in farming and those in other jobs, and those differences that were specifically associated with having been employed as a farmworker in the United Arab Emirates for at least two years.

Representativeness of samples

The study design led to a remarkably high response rate in the exposed and in both control groups. The samples should thus have been highly representative of current workers in these three categories, although those who had left their jobs, perhaps because of their health, were not represented.

Exposure assessment

As the exposed group consisted of current workers who had been in their present jobs for at least two years it is assumed that they had experienced both chronic and recent exposure. No data are given as to whether any of them had previously experienced acute poisoning, although it is mentioned that manifestations of acute toxicity were not commonly seen. Exposure status was verified by examining acetylcholinesterase activities and haemoglobin-adjusted acetylcholinesterase activities, confirming that activities in the two controls groups were similar while those in the exposed were lower. The authors state that health and safety measures were frequently disregarded by the migrant farm workers, who were often unable to read the instructions on the pesticide containers. Thus, although no quantitative measures of exposure were constructed, exposure levels might be expected to have been higher than among those doing similar jobs in other countries. No attempt was made to distinguish between different types of OP.

Organophosphates

Health outcomes

The occupational physicians who examined the study subjects were "blinded" as to their exposure status, thus reducing the likelihood of bias in the assessment of health outcomes, and the authors state that many of the exposed subjects would have been unaware of any possible risks of exposure. Highly significant differences between the three groups were found for the prevalence of twelve of the thirteen symptoms reported and in all twelve the difference was due to a raised rate among the established farm workers.

Control of potential confounders

The non-agricultural referents were matched to the established farm workers for both age and nationality, but the new farm workers were unmatched and so there may have been some scope for confounding in comparisons between them and the other groups. There may also have been confounding due to differences in the educational levels of the referents and the farm workers which were not controlled for.

Interpretation

Whilst it is not clear whether information on other symptoms was also obtained for which exposed farmers did not experience increased rates and which were therefore not reported, overwhelming evidence is presented that the exposed farmers were experiencing considerable symptomatic ill-health compared with the control groups. It seems likely that the health differences reported between the three groups were caused by occupational exposure, either to OPs or to some other substance present in or aspect of the local farming environment. The differences could have been due either to recent exposure or have been a late effect of chronic exposure, or a mixture of the two.

Hawton K, Simkin S, Malmberg A, Fagg J, Harriss L. *Suicide and stress in farmers*, London: The Stationery Office, 1998

This report describes a programme of research to investigate factors that contribute to the increased risk of suicide in farmers in England and Wales and to identify potential strategies for prevention. The programme was based on four studies. It included an in-depth study of a sample of farmers who had died by suicide (psychological autopsy study) and a study of the geographical distribution of suicides in farmers during the period 1981 to 1993. Exposure to OPs was one of the risk factors examined.

Psychological autopsy study

Study population

Data were obtained from the Office of National Statistics on farmers in England and Wales who had died between October 1991 and December 1993 with a verdict of suicide or an open verdict recorded at a coroners' inquest. Some additional cases in 1994 came directly from a coroner. The final study sample consisted of 84 cases with 71 suicide verdicts and 13 open verdicts. A retrospective profile of each of the deceased was compiled using documentary evidence from coroners' records and medical (general practitioner and hospital) files and, in particular, through interviews with relatives. In 58 of the cases the farmer was actually working in farming at the time of death; the other 26 were retired farmers. The age range of the working sample was 20 to 73 years (mean 50.2), that of the retired sample was 49 to 74 years (mean 68.4). Fifty six (97%) of the subjects in the working sample and all subjects in the retired sample were male. The mean numbers of years in farming for the working sample was 31.7.

A control group was selected from membership lists of the National Farmers' Union (NFU) and the Farmers' Union of Wales. Questionnaires were sent to 800 randomly selected members of the NFU and 200 randomly selected members of the Farmers' Union of Wales, with reminders to non-responders. A total of 500 responses was received from 974 farmers (26 having indicated that they were no longer in farming). The age range of controls was 27 to 88 years (mean 52), and 473 were male. The mean number of years in farming was 31.1.

Type of farming

Pig farming was significantly more common in the working sample (suicide cases) than in the control group (8/47, 17% *versus* 36/500, 7.2%; odds ratio, OR = 2.6; 95% confidence interval, 95%CI, 1.2-6.1). Beef farming (18/47, 38.3% *versus* 266/500, 53.2%; OR = 0.6; 95%CI 0.3-1.1) and arable farming (15/47, 31.9% *versus* 229/500, 45.8%; OR=0.6; 95%CI 0.3-1.1) were less common in the working sample but the differences did not reach statistical significance. The proportions of sheep farmers in the working sample, i.e. suicide cases, (19/47, 40.4%) and the control group (217/500, 43.4%) were similar.

Use of OPs

Eleven sheep farmers were included in the interview sample. Ten of these had used OP sheep dips and one had been drenched in the year before death but with no reported ill-effects. There was a higher rate of use of sheep dip in the working sheep farmers who had

Organophosphates committed suicide than in sheep farmers in the control sample but this difference was not statistically significant (OR =7.1; 95%CI 0.9-56.4). The proportions of all the working farmers in the suicide and control groups with health effects which they or their families attributed to OPs were similar (9.1% versus 10.3%).

Study of the geographical distribution of suicides in farmers 1981-1993

Study population

Data were obtained from the Office for National Statistics on all deaths in farmers aged 17 years and over in England and Wales between 1981 and 1993 in which a verdict of suicide or death from undetermined cause had been recorded. The information included the farmer's county of residence. In England, farmers were allocated to regions according to the classification of regions used by MAFF in the Digest of Agricultural Census Statistics 1993. The population density of farmers in each county was calculated and the distribution of types of farming in each county identified.

719 deaths were reported in this period. 634 had a verdict of suicide and 85 an open verdict. 97.6% of the farmers were male. The age range was 17 to 74 (mean 52.5) years. Of the 719 deaths, 596 were in England and 123 in Wales. The mean annual rate of suicides and open verdicts per 100,000 over the study period was 26.77 (95%CI 24.85-28.89) in England and Wales

Regional suicide rates in England

None of the regional rates of suicide in England differed significantly from the mean annual national rate (26.39, 95%CI 24.32-28.60). However, relatively higher rates were found for East Anglia (30.91, 95%CI 23.53-39.87) and the West Midlands (28.19, 95%CI 22.39-35.04). The South West region had the highest actual numbers of deaths (n=137).

County suicide rates in England & Wales

Analysis of the geographical variation in suicide rates did not show significant heterogeneity between counties (p=0.312). Relatively high rates of suicide (with relatively narrow confidence intervals) were found in Powys, Cambridgeshire, Warwickshire, Devon, Hampshire, Suffolk, West Yorkshire, West Sussex and Humberside. The 62 deaths recorded in Devon represented more than double the number of farming suicides that occurred in any other county, which reflected, in part, the large population of farmers in the county. However, the relative risk based on the mean annual rate for this county compared with the rest of England was 1.48 (95%CI 1.13-1.95). The three counties closest to Devon, i.e. Cornwall and the Isles of Scilly, Somerset and Dorset, had relatively low mean annual suicide rates.

There was no clear relationship between the mean annual suicide rate in farmers by county and the distribution of types of farm holdings.

Critique

Adequacy of control group

For the geographical study the comparisons between different regions and counties appears valid. For the psychological autopsy study the random selection of NFU members as controls could have given rise to bias if the farming practices of farmers who are members differ from those who are not. Also, the response rate from the controls was only just over 50%.

Representativeness of sample

For both studies, data on all relevant deaths in England and Wales during the years concerned were examined. Thus the entire population was involved for these years and no sampling was involved.

Exposure assessment

For most of those included in the study, the information available on exposure was limited to the type of farming carried out. More detailed information was available on those interviewed but the interview sample included only 11 sheep farmers so that the power for exposure-related comparisons within this group was limited.

Health outcomes

The selection of all deaths with a verdict of suicide or an open verdict appears to be a valid outcome measure and not subject to bias.

Control of potential confounders

There was little opportunity for the control of confounders in the analysis.

Interpretation

These studies provide reasonable evidence that exposure to OP sheep dips does not explain the excess of suicide in farmers in England and Wales.

Organophosphates

Jager KW, Roberts DV, Wilson A. Neuromuscular function in pesticide workers. Br J Ind Med 1970; 27: 273-278

In this study electromyography was used to study groups of workers with varying degrees of exposure to OP pesticides and to compare them with a control population.

Study population

Sixty six workers in a Dutch factory involved in the manufacture and formulation of pesticides were divided into three groups. These comprised, Group 1: 36 individuals who were exposed to both OP and organochlorine pesticides in the formulation plant, Group 2: 24 workers who had been exposed to organochlorine pesticides for prolonged periods but not OPs, and Group 4: six workers who were not usually exposed to pesticides but who had received an acute exposure to an OP pesticide formulation and were examined a few hours after exposure, and again 48 hours after exposure. Twenty-eight workers from a nearby oil refinery who had never been occupationally exposed to pesticides formed a control group, Group 3.

Exposure

No data were reported on exposure levels nor on the identify of the OP pesticides to which the workers were exposed, apart from a statement that they were dimethyl phosphate esters. Whole blood acetylcholinesterase activity was measured in all the workers at the time of the study. In all cases except one the levels were in the normal range.

Electromyography

Electromyographic recordings (EMG) of action potentials from the adductor pollicis muscle were made following stimulation of the ulnar nerve four times at one quarter second intervals using surface electrodes. In some cases this was described as being done both before and after a 10-second period of voluntary activity of the adductor pollicis muscle.

It is recorded that all the workers examined had a good muscular physique and were in good physical condition with none complaining of muscular weakness. No details of any other medical examination or questioning are recorded.

Results

The EMGs obtained from men in each group were compared to those associated with overtreatment of myasthenic patients with carbamate anticholinesterase compounds (neostigmine and pyridostigmine). Characteristic features of the latter are low voltage potential with repetitive muscle activity after each stimulus, and also reduced amplitude of the first EMG potential evoked by a nerve stimulus after a short period of voluntary activity in the muscle under test. Group 3, the controls, showed no evidence of repetitive activity after each stimulus, no decrease in EMG voltage after voluntary activity, and a mean EMG amplitude of 12.0 mV (SD 1.0). Of the 26 workers in Group 2, exposed to organochlorine pesticides only, one worker (4%) showed evidence of repetitive activity and depression of the first potential after voluntary activity. The mean EMG amplitude of this group was 11.7

mV (SD 1.3). However in Group 1, the workers exposed to both organochlorine and organophosphate pesticides, a total of 17 (47%) had abnormal EMGs with repetitive activity being observed (16 workers) and/or decreases in first EMG potential after voluntary activity ranging from 3 to 13% (14 workers); the sub group of 17 had a mean EMG amplitude of 10.0 mV (SD 1.2). It was stated that this subgroup had an EMG response similar to that in mild myasthenic patients. In Group 4, the accidentally exposed, 4/6 workers (67%) showed repetitive activity 2 to 3 hours after exposure, but not 48 hours after exposure. Three of these four showed a 5 to 10% depression of the first potential after voluntary activity in the earlier recordings. The EMG amplitude of this group was reduced at 2 to 3 hours post-exposure, ranging from 8.0 to 10.5 mV. There was no evidence that the blood acetylcholinesterase activity differed between the groups. EMG recordings of 6 of the 17 affected workers in Group 1 were made both before and after a five day period of exposure to organophosphate pesticides. Each worker showed either a static (1 individual) or reduced (5 individuals) EMG amplitude after the fifth day.

Critique

Adequacy of control group

Little information is given about the characteristics of the control group or the method by which they were selected, other than that they were oil refinery workers who had never been occupationally exposed to pesticides. However, it is unlikely that the method of selection would have given rise to important bias.

Representativeness of sample

As for the control group, little information is given about the characteristics of the exposed individuals other than their exposure, so that their representativeness cannot be assessed.

Exposure assessment

Few details are given.

Health outcomes

EMGs were assessed 'blind' to exposure status. The differences associated with OP exposure appear clear-cut.

Control for potential confounders

Little attention is paid in this paper to the possibility of confounding, but it is unlikely to have been a major problem for the health outcomes assessed.

Interpretation

This study suggests strongly that exposure to OP compounds can produce detectable impairment of neuromuscular function in the absence of a measurable effect on blood acetylcholinesterase activity. However, it does not indicate whether this is a long-term or only a short-term effect.

Organophosphates

London L, Myers JE, Nell V, Taylor T, Thompson ML. An investigation into neurologic and neurobehavioral effects of long-term agrichemical use among deciduous fruit farm workers in the Western Cape, South Africa. Environ Res 1997; 73: 132-145

A cross-sectional study to investigate performance in neuropsychological tests and in tests of vibration sensation in pesticide applicators in Western Cape Province, South Africa, during the peak spraying season (a study of neurological symptoms in much the same population was reported separately: see the summary of London *et al.,* 1998 below).

Study population

The exposed population consisted of current pesticide applicators working on deciduous fruit farms belonging to three large co-operatives. One farm worker not involved in pesticide spraying was selected as a control subject for every two spray men selected. They were matched for age and educational status. After various exclusions the final study sample comprised 163 spray operators and 84 controls.

Exposure to pesticides

No details are reported regarding exposure to specific OP pesticides.

Recent occupational exposure was assessed by questionnaire and from measurement of plasma cholinesterase activity with samples taken on the same day as medical examination and interview. This was stated to be within 10 days of the neuropsychological assessment. Long-term exposure to OP pesticides was estimated using a job-exposure matrix specially developed for the study, which took account of detailed occupational histories, farm records and secondary industrial data to weight job days for exposure to OPs.

Neurological evaluation

Each individual was subjected to the WHO Neurobehavioural Core Test Battery (NCTB) comprising a range of information processing tests designed for use in subjects with little education. These covered tests for the following domains – dexterity, clerical speed, visuospatial function, motor speed, encoding, apprehension, attention, semantic access, stimulus resistance, and active and passive short-term memory. Two of the NCTB screening tests covering profile of mood states and the subjective symptoms questionnaire were excluded because of poor previous performance in South African workers who had little formal education.

In addition, vibration sense was measured in the big toe of the non-dominant leg using the method of limits.

Results

Multiple linear regression analysis of the results of the NCTB screening tests showed no evidence for any relationship with cumulative OP exposure except in three tests. There was a small association with cumulative OP exposure in the Pursuit-Aiming ($p=0.018$) and

the Santa Ana (non-dominant hand) subtests (p=0.020) also with a reaction time measure of semantic memory access (p=0.026).

There was no evidence for any relationship between long-term OP exposure and loss of vibration sense.

Critique

Adequacy of control group

The occupational characteristics of the non-exposed control group are unclear, but they appear to have been comparable in all important respects other than exposure status.

Representativeness of samples

Response bias is unlikely in this study, as there was complete participation of workers in those farms that agreed to take part. It is possible that the non-participating farms, which tended to be smaller and more remote, could have had inferior working conditions including higher OP exposures, but this would at most affect the generalisability of the findings, not their validity.

Exposure assessment

The job-exposure matrix (JEM) may not have provided an accurate measure of OP exposure and, as the authors state, this remains a possible explanation of essentially negative findings.

Health outcomes

The quality of neuropsychological testing appears to have been very good.

Control of potential confounders

The most important factors were assessed and taken account of in the analysis.

Interpretation

As there were few associations found between any measure of OP exposure and any of the outcomes, these could well have been random findings that resulted from the large number of comparisons that were made.

Organophosphates

London L, Nell V, Thompson M-L, Myers JE. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense, and tremor among South African farm workers. Scand J Work Environ Health 1998; 24: 18-29

A cross-sectional study to investigate neurological symptoms, vibration sense and tremor in pesticide applicators in Western Cape Province, South Africa, during the peak spraying season (January – March) in 1993.

Study population

The exposed population consisted of pesticide applicators working on deciduous fruit farms belonging to three large cooperatives. A total of 164 applicators agreed to participate (68% of the eligible workers). The control group consisted of 83 non-spraying referents. All subjects were male coloured farm workers. The controls were group matched for age and educational status, and alcohol consumption was similar in both groups. When considering possible confounders it was noted that in both groups there was a high prevalence (71% exposed group and 70% controls) of episodes of brain injury in the past causing loss of consciousness: 34% of the exposed group and 30% of the controls had suffered loss of consciousness for 1 hour or more.

Exposure to pesticides

No details were reported about exposure to specific pesticides.

Long-term exposure estimates were based on a job-exposure matrix for agricultural chemical exposure (described in detail in London L, Myers JE. Use of a crop and job specific exposure matrix for retrospective assessment of long-term exposure in studies of chronic neurotoxic effects of agrichemicals. Occup Environ Med 1998; 55: 194-201). This entailed giving job weightings for all likely exposures for both direct exposure (dilution, application) and indirect exposure (e.g. spray drift) using data derived from industrial hygiene assessments and expert opinion. Cumulative OP exposure in kg was estimated as 3.90 (range 0.01 to 63.71) in the exposed and 1.03 (range 0.00 to 28.95) in the controls. Recent occupational exposure was assessed by a questionnaire. A total of 47% of the applicators had applied OPs in the 10 days before the examination, with 22% having done so on the morning of, or the morning before, the examination. Because of poor agreement between the estimate of acute exposure levels based on farmers' records and that reported by the subject, plasma cholinesterase measurements were used as a primary marker of recent exposure. These were comparable in the exposed and control groups.

Neurological evaluation

Neurological symptoms were assessed from a check list that included twelve presumptively neurological and two dummy symptoms not indicative of neurotoxic effects, namely ear ache and chest pains. All the reported symptoms were qualified by answers to an additional question on chronicity, defined as presence for the past three months.

Vibration sense was measured in the big toe of the nondominant leg using the method of limits and the vibrotactile thresholds were identified.

Static motor steadiness was measured by investigating the ability to hold a stylus pen inside holes in a metal plate of decreasing diameter for 15 seconds without touching the sides. Dynamic steadiness was measured by negotiating the pen around a maze mounted on a board at 45° without touching the sides of the maze.

Results

Current pesticide applicators reported significantly more dizziness, sleepiness and headache ($p<0.05$); in all cases significant excesses were seen for both current and persistent symptoms. The pesticide applicators had a higher overall neurological symptom score ($p<0.005$). This association remained statistically significant in a multiple logistic regression analysis controlling for a range of confounders. There was no significant increase in the dummy symptoms in the exposed group. Increased reporting of neurological symptoms was particularly associated with past pesticide poisoning (OR 4.08, 95%CI 1.48-11.22).

No differences were seen between the exposed and the control groups in the tests of vibration sense or motor steadiness.

Critique

Adequacy of control group

The referents were well selected, from the same farms but in occupations not exposed to pesticides. The groups were well matched.

Representativeness of samples

Approximately half the eligible subjects participated. The pattern of non-response, about half of which involved non-participation of whole farms, suggests that response bias may have been less important than this overall figure would suggest. However, the exclusion of smaller farms may have led to workers with higher exposures being omitted.

Exposure assessment

Blind assessment was carried out, the exposure assessors and the health outcome assessors being separate. Long-term exposure was determined using a specially designed job-exposure matrix. Recent exposure was available from questionnaires, farm records and using plasma cholinesterase activity, but these sources disagreed (plasma cholinesterase activity was used in the analyses).

Health outcomes

Symptom reporting was associated both with current exposure and with a history of past poisoning, presumed from indirect evidence to be due usually to OPs. Symptoms were not related to long-term OP exposure in the absence of poisoning. The more objective health

Organophosphates outcomes were not associated with any indices of exposure, except that the five workers with clinical neurological deficits were all in the current applicator group (a non-significant finding statistically).

Control of potential confounders

Technically the adjustment process was good, but the high levels of alcohol consumption, life-long poverty and malnutrition, and the frequency of previous head injuries mean that only partial control may have been achieved.

Interpretation

Good use was made of "dummy" symptoms to try and distinguish true effects from reporting bias. A 10 percent repeatability sample also enhanced the study (although correlation coefficients were inappropriately used for analysing the continuous variables). A comparatively large population was studied, with well-matched controls, and the use of blinding was a further advantage. However, the study had the limitations of a cross-sectional design with exclusion of any workers too ill to remain at work and the substantial level of socio-medical disadvantage in this population limits the generalisability.

The study confirms the association of neurological symptoms both with current exposure and with a history of past poisoning. The absence of associations with long-term exposure could be due to an inaccurate job-exposure matrix, but the results give no support to a long-term effect of OP exposure on the neurological outcomes studied other than after poisoning episodes.

Maizlish N, Schenker M, Weisskopf C, Seiber J, Samuels S. A behavioral evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. Am J Ind Med 1987; 12: 153-172

This was a study to investigate neuropsychological effects in pesticide (diazinon) applicators using pre- and post-shift data and comparison with controls.

Study population

The exposed population consisted of California state employees involved in the third year of a programme to eradicate Japanese beetle from a small area of Sacramento Country California in 1985 (n = 46, both male and female). Controls (n = 53, both male and female) were recruited either from county personnel involved in pest detection and inspection of agricultural commodities or were non-exposed supervisors of the diazinon applicators. The response rate in both groups was 56%.

Differences were identified between the applicators and the control group in respect of age, medical history, consumption of alcohol or caffeinated drinks, and use of tobacco or pesticides at home.

Exposure

The exposed group applied diazinon granules using a variety of methods, mainly to lawns. It was stated that appropriate protective clothing was worn, namely disposable coveralls, rubber boots and gloves; in addition when loading diazinon into spreading equipment, a face shield and full face respirators were used. The mean duration of such pesticide application at the time of the neurobehavioural testing was 39 (SD 12) days.

Urinary measurements of the diazinon metabolite diethylthiophosphate (DETP) were carried out pre- and post-shift. In the case of the applicators mean concentrations (± SD) were 4 ± 3 µg/g creatinine and 9 ± 4 µg/g creatinine respectively. In the case of the control group no increase in DETP concentrations was seen post-shift, with values of 2 ± 3 µg/g creatinine being recorded at both times.

Quantitative data on full shift exposures at the time of testing were available for about 20% of the study population from personal air monitors and passive skin badges. Mean (± SD) values of 1.5 ± 4.9 mg (range 0.1 to 10.4) were obtained for the applicators (n = 10) and 0.02 ± 0.006 mg (range 0.002 to 0.2) for the controls.

Neurological tests

During a single day, the subjects completed pre- and post-shift neuropsychological testing involving a battery of seven tests. These were selected on the basis of sensitivity, reliability, ease of administration, subject acceptance, and inclusion of CNS functions known to be affected by pesticide exposure. They included a continuous performance test measuring complex reaction time (attention/vigilance), a hand-eye coordination task (visual/motor accuracy), a symbol-digit substitution task (visual/motor speed), a pattern comparison test

(visual perception), a pattern memory test (visual memory), an AFQT (Armed Forces Qualifying Test), vocabulary test (verbal ability), and a finger tapping test (motor speed).

The subjects also completed a questionnaire concerning their work and medical history and were given a neurological examination which included evaluation of cranial nerves, pupillary reaction, extraocular movements, nystagmus, visual fields and acuity, tremor, reflexes, coordination and gait, and an overall impression. The purpose of this physical examination was to identify individuals whose neuropsychological test performance may have been affected by pre-existing factors unrelated to pesticide exposure.

Results

Analysis of the results from the neuropsychological screening battery both pre- and post-shift by multiple linear regression models with adjustment for age, sex, education and alcohol intake revealed few differences between the exposed group (applicators) and the controls.

In the symbol digit test applicators were slower than non-applicators in matching symbol digit pairs in the pre-shift session by 4% (p=0.08) and in the post-shift session by 9% (p=0.02) after adjusting for confounders. In the pattern memory test no significant differences were found between applicators and non-applicators in speed of recall of patterns in the pre- and post-shift sessions. However, applicators correctly identified fewer patterns than non-applicators in the pre-shift and post-shift tests (p=0.07). Statistically significant (p=0.03) differences were seen in post-shift performance adjusted by pre-shift performance. Finally, in the finger tapping test (left, right and alternating), performance was not significantly associated with exposure. Some conflicting data were reported. Significantly slower (p=0.03) tapping with the right index finger was found amongst the applicators in the post-shift session when adjusted for pre-test performance. However, faster alternate tapping was found amongst applicators adjusted for pre-test performance (p=0.04).

There were no changes in the neurological test battery in relation to urinary DETP levels.

The prevalence of 18 symptoms considered as being possibly related to diazinon exposure was not elevated in the exposed group.

Critique

Adequacy of control group

The non-exposed control group was different from the exposed group in many respects, some of which could have acted as positive confounders (e.g. applicators had less education and drank more alcohol) while others could have acted as negative confounders (e.g. they tended to be younger and reported fewer medical problems). Biochemical measurements established that the control group were subject to very low exposures.

Representativeness of samples

The sampling frame appears to have been adequate, and the response rate was 56 percent in both groups. Thus there is some possibility of response bias.

Exposure assessment

The exposure was solely to diazinon (used in granular form). Measurements of urinary metabolites were made pre- and post-shift for both groups, and in addition whole body exposure was estimated in 20 percent of individuals using a variety of methods. The level of exposure was low, partly because precautions were made to minimise it.

Health outcomes

Any observed differences were small and mostly not statistically significant. On a number of tests, applicators were poorer than controls in both pre- and post-shift testing; however, multiple regression analyses using duration of previous exposure (in days) did not suggest that this was due to the effects of cumulative exposure. Reports of relevant symptoms during the previous month were not elevated in applicators.

Control of potential confounders

This was probably adequate.

Interpretation

The controlled, before/after design was a strong one for assessing the short-term effects of exposure, but the study does also provide information about longer term risk since the applicators had been exposed during the previous month, and some had had exposure to other (more toxic) OPs in previous seasons. The levels of exposure were generally low but the findings give little indication that they influenced health in the long term. It is possible that a risk of severe disease was missed because the study was restricted to people currently in work.

Organophosphates

McConnell R, Keifer M, Rosenstock L. Elevated quantitative vibrotactile threshold among workers previously poisoned with methamidophos and other organophosphate pesticides. Am J Ind Med 1994; 25: 325-334

This was a study to investigate peripheral neuropathy following acute poisoning with OPs including methamidophos in Nicaragua.

Study population

The exposed population was selected from all patients with a discharge diagnosis of pesticide poisoning over the period between 1st July 1986 and 31st July 1988 from a teaching hospital in Nicaragua. A total of 52 male patients were identified. Of these 38 were located and invited to participate (the remainder could not be included because their addresses were unknown or they had moved too far away); 36 (95%) agreed to participate.

Each was matched by sex and age (within 5 years) to a sibling or friend from the same community who had never been treated for pesticide poisoning.

Exposure

All the exposed group had been admitted to hospital for occupationally-related OP intoxication and all had received atropine treatment. In 21 out of the 36 individuals the poisoning was stated to be due to methamidophos. In the remainder it was due to other OP pesticides but no details were given.

Neurological examination

Neurological examination, using vibrotactile thresholds as an index of peripheral neuropathy, were carried out between 10 and 34 months after admission to hospital for acute OP poisoning. The vibrotactile measurements were carried out using both right and left index fingers and right and left big toes.

Results

Subjects were divided into three groups for analysis of the data; never poisoned (n=35); poisoned by an OP pesticide other than methamidophos (n=15); and poisoned by methamidophos (n=21). A statistically significant trend of increasing vibrotactile threshold (age and height adjusted) was seen across these groups for all the four digits examined. The p values for this trend were as follows: right index finger 0.007, left index finger 0.002, right first toe 0.001, left first toe 0.003. In all cases the greatest increase was seen in the group known to have been poisoned by methamidophos.

Critique

Adequacy of control group

The use of a close male friend or relative can be criticised, but was probably the best available option in the circumstances of the study. Controls were similar to the previously

poisoned men in age, slightly better educated and tended to consume a little more alcohol (differences were not statistically significant). Two-thirds of them had worked with pesticides at some time, and the previous exposure to pesticides was similar to that of the previously poisoned men.

Representativeness of samples

The group of previously poisoned men invited to take part in the study represented 38 out of 52 eligible subjects, the other 14 not having been traced. Among the 38, the response rate was 95 percent (36 out of 38).

Exposure assessment

Eligibility was based not on exposure but on the prior experience of having been admitted to hospital for acute OP poisoning. The diagnosis and information about pesticide exposures were obtained from hospital records supplemented by a questionnaire. Of the 36 subjects, 14 were recorded as having been poisoned with methamidophos, a known peripheral nervous system toxicant, and seven more reported having been treated for methamidophos poisoning.

Health outcomes

Vibrotactile thresholds were higher in those 21 with recorded or reported methamidophos poisoning than in the other 15, and these in turn had higher vibrotactile thresholds than the control group.

Control of covariates

Exclusion of those with pre-existing disease from the study should have ensured that concurrent illnesses such as diabetes did not confound associations. Alcohol consumption was not a confounding factor in the comparison between OP poisoned men and controls. Within the former group, alcohol intakes for those poisoned by methamidophos and other OPs were not reported separately. However, alcohol was not associated with higher vibrotactile thresholds in this study. Solvent exposure tended to be higher in the previously poisoned group.

Interpretation

Despite the limitations of the design, this study provides strong evidence of long-term elevation of vibrotactile thresholds in individuals occupationally exposed to OPs who had previously been treated for an acute poisoning incident. This was more marked after methamidophos poisoning, but was also seen in those poisoned with “non-neuropathic” OPs.

Organophosphates

Misra UK, Nag D, Khan WA, Ray PK. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. Arch Toxicol 1988; 61: 496-500

This was a study to investigate effects on nerve conduction and neuromuscular function in a small group of OP pesticide sprayers in India who used no protective clothing.

Study population

The exposed group consisted of 24 workers engaged in regular spraying using the OP fenthion, and, to a more limited extent, Paris Green (copper acetoarsenite). Their mean age was 31.7 years (range 22 to 52) and their mean duration of exposure to the OP was 8.5 years (range 1 to 19). The control group consisted of 19 hospital employees matched for age, sex, socioeconomic and nutritional status, who had no direct exposure to pesticides.

Exposure to the OP fenthion

The exposed group sprayed formulations of the pesticide for 5 to 6 hours daily without using any protective clothing. The average quantity of fenthion sprayed was in the range 1500 to 2000 litres per month.

Serum cholinesterase activities were measured after spraying and following three weeks withdrawal from exposure. Activities were significantly depressed ($p<0.01$) immediately after spraying (by about one third), but had increased to near those of the controls after the three week withdrawal period.

Neurophysiological and other tests

Workers were examined using a range of clinical and neurophysiological tests on the day after spraying. These included measurement of maximum motor conduction velocity of the median and peroneal nerves and of sensory conduction velocity in the median and sural nerves, F response by recording of F wave from abductor pollicis brevis by stimulation of median nerve at the wrist, H reflex evaluation by recording of H reflex (late response) by stimulation of the tibial nerve, and a repetitive nerve stimulation test using the MS VI electromyograph. In addition, they were questioned about their medical and occupational history and physically examined.

Follow-up neurophysiological studies were carried out after withdrawal of the workers from exposure to fenthion for three weeks.

Results

After spraying fenthion the workers reported a number of minor symptoms including headache (58%) giddiness (50%) ocular symptoms (29%) and paraesthesiae (17%). The ankle jerk reflex was absent in two pesticide workers.

Regarding the neurophysiological tests, comparison of the pesticide workers on the day post-exposure with the control group showed no statistical difference in any of the parameters measured (motor and sensory nerve conduction velocity F response, H reflex evaluation). No decrement was seen in the repetitive stimulation test. Repetitive activity was seen in 7 (29%) of the sprayers.

However, significant differences were seen in the exposed workers after the three week withdrawal period compared to the day immediately post-exposure. This included an increase in peroneal motor conduction velocity ($p<0.05$), a decrease in terminal motor latency of median ($p<0.1$) and peroneal nerves ($p<0.05$), together with effects on F minimal latency ($p<0.01$) and H reflex latency ($p<0.01$). The repetitive activity noted in seven workers the day after fenthion exposure was not present after the three week period of no exposure.

Critique

Adequacy of control group

Two comparisons are made, firstly a comparison between occupationally-exposed agricultural workers and hospital employees, and secondly an internal comparison within the group of agricultural workers in which observations taken on the day following a working day were compared with those following a three week withdrawal period. There may well have been some differences between the agricultural workers and the hospital workers other than exposure to OPs, but the internal comparison should not have been confounded.

Representativeness of sample

No information is given on how the sample was selected.

Exposure assessment

The exposed group were all known to have worked intensively with fenthion without protective clothing and were known also to have been exposed to Paris Green. Confirmation of exposure status was available from serum cholinesterase activities.

Health outcomes

No mention is made of whether the assessor was aware of an individual's exposure status in the assessment of the health outcomes. Thirteen tests of significance were carried out and, for the internal comparison, five significant differences were found compared with fewer than one expected by chance alone.

Control of potential confounders

For the internal comparison no covariate adjustment should have been necessary.

Interpretation

The internal comparisons in this study provide clear evidence of changes in several neurophysiological measurements in pesticide spraymen on the day following spraying compared with measurements made on the same workers after a three week withdrawal period. However, the study does not support a long-term influence of OP exposure on peripheral nerve or neuromuscular function. Because the study was restricted to subjects who were in work, it would not have detected health effects that were sufficiently disabling to prevent continued employment.

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Otto DA, Sollman S, Svendagaard D, Soffar A, Ahmed M. Neurobehavioral assessment of workers exposed to organophosphorus pesticides. In: *Advances in neurobehavioral toxicology: applications in environmental and occupational health*, edited by Johnson BL, Anger WK, Durao A, Xintaras C. Chelsea, Michigan: Lewis Publishers, 1990, pp. 306-322.

A cross-sectional study of workers from a pesticide factory using neuropsychological and neurological tests for the early detection of delayed peripheral neuropathy.

Study population

The exposed group consisted of male production workers from a pesticide formulation plant in Egypt. Those workers who were scheduled for routine annual physical examination during the first year of the study (1985) were selected. These comprised 229 workers out of a total workforce stated to be 600 to 700 (i.e. about 35%). Two referent populations were used, workers from a fertiliser plant in the same city and from a textile plant in a different city in Egypt. The total numbers participating from each were 180 (out of a total workforce of 800 to 900, i.e. about 21%) and 167 (from a total workforce stated to be over 1000, i.e. below 17%) respectively.

There was a considerably higher proportion of the pesticide workers in the youngest of the three age strata distinguished in the analysis; 32% of the pesticide workers in the study were in this group compared to 17% of the fertiliser workers and 9% of the textile workers. In addition a higher proportion of the pesticide workers (9.6%) had attended college compared to the fertiliser workers (6.7%) and the textile workers (4.8%). The proportion of smokers was similar in each group.

Exposure to pesticides

It was noted that at least four OP pesticides that have been associated with OPIDN had been formulated at the plant. These were (with the years during which they had been formulated): EPN (1970 to 1983), leptophos (1968 to 1975), methamidophos (1970 to 1986) and trichlorfon (1961 to 1970). In addition the following other OPs had also been formulated: diazinon (1978 to 1986), dimethoate (1978 to 1985) malathion (1978 to 1985) and phenthoate (1986). Total output for each over the production period was in the range 1,500-6,500 metric tons, except for phenthoate, formulation of which had only started in 1986 (the final year of the study).

It was stated that the workers at the fertiliser plant were not exposed to pesticides, but that they were exposed to two chemicals that gave rise to some concerns regarding neurological effects, namely sulphuric acid (claimed by the authors to be neurotoxic) and lead. No neurotoxic chemicals were used in the textile plant.

Serum cholinesterase activities (and also lymphocyte neuropathy target esterase activities) were measured in all groups during both years of the study (1985 and 1986). Median serum cholinesterase activities were lower in the pesticide workers. Values (± SD) reported in 1986 were 2064 ± 588 mU for the pesticide workers compared to 2299 ± 531 mU in the

fertiliser workers and 2389 ± 402 mU in the textile workers. Similar values were obtained in 1985. The lymphocyte neuropathy target esterase activities were also lower in the pesticide formulators.

Neurological tests

Each worker answered a questionnaire designed to obtain demographic data, workplace hygiene information, general health history and data relating specifically to neurological effects. They were subjected to a ½ hour battery of psychometric tests, tactile sensitivity assessment and neurological examination. Specifically these included examination of memory, coordination, tremor, ankle jerk, sensation and objective recall; measurement of tactile sensitivity; the block design test, (subtest subgroup of the Wechsler Adult Intelligence Scale) to measure ability to perceive spatial relationships; and the Santa Ana dexterity test for hand-eye coordination and motor performance.

Studies were carried out in both 1985 and 1986. The pesticide and fertiliser groups were tested by persons blind to their exposure status, but not the textile workers.

Results

In view of the fact that the textile workers were not tested blind, data from this group were included only in the analysis of the objective end-points (tactile sensitivity, block design and Santa Ana dexterity tests).

The pesticide workers showed clear excesses as compared with the fertiliser workers in a number of symptoms that could be related to OP exposure. These were numbness in legs and feet (39% *versus* 23%), pain in both legs (33% *versus* 19%), numbness in hands (34% *versus* 5%), pain in both hands (30% *versus* 4%), memory difficulty (29% *versus* 16%), stomach trouble (21% *versus* 13%) and frequent tiredness (37% versus 28%). There was no marked difference in other symptoms.

Regarding the neurological examination it was stated that workers in the pesticide and fertiliser plants had more neurological impairment than did textile workers on most tests. In three tests (hand coordination, involuntary tremor and knee jerk) the difference between plants was stated to be quite dramatic, but no data were given. Also, testing of the textile workers was not blind and statistical comparisons were made only between the pesticide and fertiliser workers. The only differences seen after adjusting for age and education, were an increase in abnormal vibration sense ($p<0.0001$) and involuntary tremor ($p=0.05$) in the pesticide workers. No effects were noted on the block design tests or the Santa Ana dexterity tests.

Tactile sensitivity was stated to be the most sensitive index of neurotoxicity in the pesticide workers, and decreased more rapidly with age in the pesticide workers than in other groups. It was noted also that diabetic individuals were identified as having the highest tactile threshold of any workers except the oldest pesticide workers (peripheral neuropathy being a recognised complication of diabetes mellitus).

Organophosphates **Critique**

Adequacy of control group

The fertiliser factory workers were comparable to the pesticide workers in most respects. Any biases in comparisons of the two groups are likely to have been conservative, because (a) the fertiliser group were themselves exposed to a potential neurotoxicant (lead), and (b) the pesticide group tended to be younger and better educated. Comparisons with the textile workers are less useful, because the groups tended to be substantially different and observations were not blinded.

Representativeness of samples

The precise sampling frame was not specified, and it is therefore not possible to assess the potential for response bias. Workers were selected on the basis of having been scheduled for routine annual physical examination during the period of the study, which suggests that this may be less of a problem than the participation figures suggest.

Exposure assessment

Exposure to OPs among the pesticide workers was confirmed by measuring activities of serum cholinesterase and lymphocyte neuropathy target esterase.

Health outcomes

It is not stated how many observers carried out the neurological examination, and the results of this are not clearly presented.

Control of potential confounders

This appears to have been satisfactory.

Interpretation

This study provides evidence of peripheral nerve impairment including sensory abnormalities in workers exposed to OPs.

Summaries and critiques of the epidemiological literature

Pickett W, King WD, Lees RE, Bienefeld M, Morrison HI, Brison RJ. Suicide mortality and pesticide use among Canadian farmers. Am J Ind Med 1998; 34: 364-372

This was a case-control study to investigate the link between exposure to pesticides and suicide in Canadian farmers.

Study population

The study population was drawn from the Canadian Farm Operatives Cohort (CFOC, Health/Statistics Canada) comprising 326,256 men. Cases (of which there were 1457) were those who had died over the period 1971 to 1987 with the death being classified as suicide. For each case eight controls were identified, who were alive at the time of the death of the case; they were matched for age and province of residence. The total number of controls was 11,656.

Exposure

Three categories of exposure to pesticides were identified from information provided in the Canadian Census of Agriculture and the short Census of Population questionnaire. These were: acres sprayed with herbicides; acres sprayed with insecticides; and costs of agricultural chemicals purchased. Each was graded into four levels from zero to highest. No data were available on exposure to specific pesticides or exposure levels.

Results

Multivariate logistic regression analysis provided no evidence for any association between suicide and any of the three categories of exposure, after controlling for important covariates, including some measures of socioeconomic status. The only subgroup where there was an increased risk related to farms where there was no hired help (OR 1.71 for 1 to 48 acres sprayed with herbicide and OR 1.91 for 1 to 15 acres sprayed with insecticides). These increases were not significant, and there was no dose-response in that odds ratios decreased at the higher acreage levels.

Critique

Adequacy of control group

As control subjects were selected from members of the CFOC, i.e. the cohort from which the cases were also obtained, they should form an adequate control group. An exception to this might occur if the controls were not representative of the CFOC. This could have happened if the response rate among those selected to complete the 'long census' form (in principle a random sample of one-third of cohort members) was low. No information is given on this rate.

Representativeness of sample

As the sample of those who committed suicide was obtained by linking the members of the CFOC with the Canadian national mortality database it should have been representative

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of the CFOC. Departures from this ideal situation might have occurred through misclassification of causes of death (see health outcomes below).

Exposure assessment

As exposure was assessed from information collected previously in national surveys it is unlikely to have been biased. However, it is likely to have been subject to random misclassification and also lacked detail, with no information on the extent of exposure received by individuals, or the specific insecticides involved.

Health outcomes

Death due to suicide would seem to be a valid measure, although since the cause of death was obtained from official statistics some cases may have been omitted, as there is a tendency to under-report suicide on death certificates.

Control of potential confounders

Adequate attention was given to the control of covariates in this study.

Interpretation

This study provides no evidence to support the hypothesis that exposure to OPs is a cause of death by suicide. However, the design of the study means that it would only have had a limited ability to detect such an association.

Reidy TJ, Bowler RM, Rauch SS, Pedroza GI. Pesticide exposure and neuropsychological impairment in migrant farm workers. Arch Clin Neuropsychol 1992; 7: 85-95

A study to investigate the long-term neuropsychological effects of acute and chronic exposure to pesticides in migrant farm workers.

Study population

The exposed population consisted of 21 Hispanic field workers who had experienced two documented episodes of acute toxicity arising from a combination of mevinphos (an OP), methomyl (a carbamate) and maneb (a dithiocarbamate) in 1981 and 1985. They were referred for neuropsychological screening by an attorney representing them in workers' compensation litigation relating to the two exposure incidents. A control group of 11 Hispanic workers matched for age, sex, education and socioeconomic status was drawn from a local cannery; they were recruited through the cooperation of a food packers' union and were paid.

Exposure to pesticides

The exposed subjects had experienced two episodes of treatment in an emergency room following over-exposure to the pesticides mevinphos, methomyl and maneb. They were also stated to be subject to chronic low level pesticide exposure but no further details of this were given. Symptoms of toxicity noted after the acute episodes included headaches, dizziness, visual disturbances, nausea and vomiting. Plasma and erythrocyte acetylcholinesterase activities were stated to have been within normal limits at the hospitals, although it was also stated that reactivation of acetylcholinesterase activity was documented for several of the workers who were tested on more than one occasion. No further details were given. It was stated, however, that following the second exposure five of the exposed workers were diagnosed as having peripheral neuropathy which was believed to be due to heavy or repeated exposure to mevinphos.

Neuropsychological tests

Each individual in the study sample was tested with the California neurobehavioral screening battery about two years after the second episode of acute intoxication. This included the Wechsler Memory Scale, Wechsler adult intelligence scale – revised (WAIS-R) subtests (digit span, digit symbol, alternate items of vocabulary, arithmetic, block design), Trail making test A and B, cancel H test, finger tapping test, Purdue pegboard, dynamometer and Benton visual retention test (multiple choice format). In addition, a verbal questionnaire was used to assess history of illness and mood.

Results

After correction for multiple comparisons the exposed group was shown to be significantly impaired compared to the controls on several tests. These were nondominant hand finger tapping ($p<0.009$) and Purdue pegboard dominant hand ($p<0.001$) and non-dominant hand ($p<0.005$). In addition the dominant hand finger tapping ($p<0.02$) and the Benton visual retention test (major rotations) ($p<0.03$) came close to statistical significance.

Organophosphates In the assessment of mood the exposed group scored significantly higher than the controls on the measures of anxiety ($p<0.001$) and depression ($p<0.008$).

Critique

Adequacy of control group

Although the controls were matched for age, sex, education and socioeconomic status to the exposed group, they were volunteers drawn from a population of currently employed workers and received a payment for taking part in the study. Therefore there is considerable scope for bias between them and the exposed subjects, especially in tests that required concentration and effort from the subjects.

Representativeness of sample

No information is given as to whether or not the 21 exposed workers included in the study comprised all those who received both acute exposures, or whether they formed a selected subgroup in some way. Thus the possibility that they were not representative of all those exposed cannot be ruled out.

Exposure assessment

No formal exposure assessment of the second acute exposure appears to have been carried out and records relating to the first acute exposure were not generally available. As both incidents involved several non-OP compounds as well as mevinophos, it is not possible to attribute any harmful effects to OPs with certainty.

No attempt was made to assess the extent to which the individuals were chronically exposed during the normal course of their work.

Health outcomes

The exposed group experienced problematic neurotoxic symptoms appreciably more often than controls. However, this would be expected as few individuals would go to the trouble of engaging in litigation unless they were experiencing symptoms. As regards the neurological tests five out of 19 differed significantly between the two groups at the 5% level or less using a one-sided test. This reduces to four if a more usual two-sided criterion is applied, whereas only about one significant result would have been expected by chance alone. Three of the four neurological tests were for psychomotor variables.

Control of potential confounders

The exposed group had longer work histories than the controls, but no efforts were made to correct for this in the analysis.

Interpretation

This study provides limited support for long-term neurotoxicity following overt OP poisoning, but because of the method of subject selection strong conclusions are not possible.

Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K, The Pesticide Health Effects Study Group. Chronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 1991; 338: 223-227

A retrospective cohort study to investigate neurological sequelae of acute OP poisoning of agricultural workers in Nicaragua.

Study population

The exposed population was selected from all patients from a teaching hospital in Nicaragua with a discharge diagnosis of pesticide poisoning over the period 1st July 1986 to 31st July 1988. A total of 89 male patients were identified. These were subjected to the following inclusion criteria: medical record documentation of having sustained an unintentional work-related OP pesticide poisoning incident; age 15 to 44 years and with no evidence of other serious illness or history of neurological disorder. Fifty two men were identified as being eligible for the study. Of these 38 were located and invited to participate (the remainder could not be included because their addresses were unknown or they had moved too far away); 36 (95%) agreed to participate.

Each was matched by sex and age (within 5 years) to a sibling or friend from the same community who had never been treated for pesticide poisoning.

Exposure

All the exposed group had been admitted to hospital for occupationally related OP intoxication. No details of the specific compounds involved were given. In a separate paper concerned with the same exposed cohort (summarised above, McConnell *et al.,* 1994) it was stated that 21 of the 36 had been poisoned by methamidophos, and the remainder by other OPs.

Neurological examination

Individuals were subjected to a range of neurological tests in May-June 1989. This was on average 23.6 months (range 10-34 months) after the acute poisoning episode. The period chosen for these examinations was immediately prior to the OP spraying season.

Subjects completed an occupational/health history questionnaire at the time of the neurological examination. The neuropsychological assessment consisted of six of the seven WHO core battery tests namely simple reaction time, pursuit aiming, the Santa Ana manual dexterity test, the Benton visual retention test, and the Wechsler adult intelligence scale revised (WAIS-R) digit span and digit symbol sub-tests. To evaluate potential psychiatric and affective disturbance a brief symptom inventory was substituted for the profile of mood states (POMS). Six additional Spanish translated standardised tests were also included – the Rey auditory verbal learning test, finger tapping, Trails A, WAIS-R vocabulary test, block design subtests and digit vigilance. A test to assess literacy was devised and field tested. Symptoms consistent with CNS origin were assessed by a 16 item questionnaire.

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Results

The exposed group gave a significantly higher number of positive responses in the neurophysiological questionnaire (7.2 *versus* 4.7, $p<0.01$). They also had lower levels of performance on the six subtests of the WHO core battery that assessed verbal attention, visual memory and visuomotor and motor functions, the difference being statistically significant ($p<0.01$) in five of the six subtests. In addition significantly poorer ($p<0.01$) performance was seen in the additional tests of visuomotor sequencing and problem solving: Trails A, block design and visual attention (digit vigilance).

Critique

Adequacy of control group

The use of a close male friend or relative can be criticised, but was probably the best option in the circumstances of the study. Controls were similar to the previously-poisoned men in age, slightly better educated and tended to consume a little more alcohol (differences were not statistically significant). Two thirds of them had worked with pesticides at some time and their previous exposure to pesticides was similar to that of the previously-poisoned men.

Representativeness of samples

The group of previously-poisoned men invited to take part in the study represented 38 out of 52 eligible subjects, the other 14 not having been traced. Of the 38, the response rate was 95 percent (36 out of 38).

Exposure assessment

Eligibility was based not on exposure but on the prior experience of having been admitted to Leon University Hospital for acute OP poisoning. The diagnosis and any information about pesticide exposures were obtained from hospital records supplemented by a questionnaire.

Health outcomes

The previously poisoned group did significantly less well in tests assessing a wide range of neuropsychological function, and were more likely to report symptoms consistent with central nervous system toxicity. Depression and anxiety were not increased.

Control of potential confounders

Adjustment was made for vocabulary scores, because these were found to be lower among the previously poisoned men than among controls, suggesting differences between the two groups that were independent of any possible effects of OP toxicity; this decreased the explanatory power of the variables that were significantly associated with prior OP poisoning (data not shown). The authors suggest that this may have been conservative as the vocabulary scores themselves could have been affected by the prior episode of poisoning. The possible effects of differences in socioeconomic status including educational level were also allowed for.

Interpretation

Despite the limitations of the design, the impossibility of blinding in the questionnaire part of the study, and the possibility that mild neurological abnormalities may predispose to accidental pesticide poisoning, this report provides strong evidence of long-term sequelae following acute poisoning with OPs.

Summaries and critiques of the epidemiological literature

Organophosphates

Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health 1988; 43: 38-45

An investigation of chronic neurological and neuropsychological abnormalities in subjects who had previously experienced acute OP poisoning.

Study population

Potential cases were identified from rosters of OP poisoning cases in Colorado occurring in the years 1950 to 1976 (443 cases) and in Texas in the years 1960 to 1976 (400 cases). After screening these for completeness of documentation, numbers were reduced to 303. A total of 200 (66%) could be located and these were then screened using the following criteria. For inclusion: a documented history of at least one OP poisoning incident, a physician's diagnosis that was consistent with OP poisoning, a minimum age of 16 at the time of follow-up, and an understanding of English. For exclusion: OP poisoning within 3 months of the study, diseases of the CNS including trauma-induced unconsciousness for longer than 15 minutes, learning disability or congenital defects of the CNS, and a history of alcohol, narcotic or other drug abuse. These exclusions, together with unwillingness to participate in 27, reduced the number to 100. These individuals formed the exposed population.

Each case was individually matched to a control for sex, age, socioeconomic status, race and ethnic background. The controls were obtained from Colorado or Texas; 35 were recruited through referrals from study participants, 24 through employee rosters furnished by businesses and public agencies, 37 through investigator solicitation and 4 from miscellaneous sources.

Exposure

All the exposed group had a history of at least one episode of acute OP poisoning that was confirmed by a physician. A total of 10 different OPs were listed as the primary cause of poisoning. These were methyl parathion (54), parathion (42), disulfoton (8), malathion (6), mevinphos (5), dicrotophos (2), TEPP (2) and one case each of dioxathion, *S,S,S*-tributylphosphorothioate and phorate. These numbers total to more than 100 since more than one OP was implicated in some incidents.

Neurological examination

Neurological history was recorded on standard forms by an examining neurologist. The neurological examination consisted of an evaluation of cranial nerve function, motor system function, sensory system function, and tests for cerebellar function and coordination and mental status. All subjects were examined by EEG and subjected to a range of neuropsychological tests; Wechsler Adult Intelligence Scale and expanded Halstead-Reitan battery (including measures of intelligence, attention, cognitive function, motor, proficiency and sensory-perceptual functions, aphasia and related disorders, and learning and memory), three subtests from the Peabody Individual Achievement Test and an objective personality test, Minnesota Multiphasic Personality Inventory (MMPI).

In addition, each participant and a spouse or other close relative independently completed questionnaires relating to the participant's functioning with respect to memory,

communication skills and academic skills, sensory and motor abilities, various cognitive and intellectual abilities and emotional status. It was stated that the data provided subjective ratings of the same general abilities and emotional factors that were assessed objectively in the comprehensive neurophysiological evaluation.

In addition all subjects had a physical examination and also an audiometric and ophthalmic examination.

Results

There were no significant differences between the exposed and the control group with regard to the physical examination, audiometric and ophthalmic examination and EEG.

Results from the neurological examination (over 50 tests) revealed a few differences between the groups. The exposed group had more than twice as many abnormal classifications in one portion of the memory component (three-pairs-of-items, $p=0.006$). Of 10 abnormal classifications in the abstraction category, nine were from the exposed cohort ($p=0.028$). In the mental status test six of the exposed group were classified as depressed compared to none of the controls ($p=0.003$).

More marked differences between the two groups were seen in the neuropsychological tests which covered a wide range of abilities including intellectual functioning, academic skills, abstraction and flexibility of thinking and simple motor skills. Twice as many of the exposed group (24 *versus* 12, $p=0.02$) had Halgstead-Reitan battery summary scores that were within the range characteristic of individuals with documented cerebral lesions. Of the 11 test measures that contributed to the average impairment rating the exposed group were significantly worse in three and overall showed significant impairment ($p<0.01$). Although both groups showed above average intellectual functioning in the WAIS, the exposed group had a mean full scale IQ that was approximately 5 points lower than the control mean ($p<0.001$). The exposed group did significantly worse than the controls on components of the Peabody Individual Achievement Tests namely: reading recognition ($p=0.001$), reading comprehension ($p=0.008$) and spelling sub-test ($p=0.004$). They also did significantly worse than controls on all six verbal sub-tests and on one of the five performance sub-tests. Although the mean scores in the MMPI were within normal limits there were significant differences between the groups relating to validity ($p=0.008$), defensiveness ($p=0.018$), paranoia ($p=0.027$) and social introversion ($p=0.050$). The overall difference between the exposed and control groups was highly significant ($p=0.008$).

Significant differences between the exposed and control groups were also noted in the questionnaires completed by each participant. Specifically, the exposed group reported more difficulties in understanding speech of others ($p=0.014$), in recognising words ($p=0.014$), in thinking of names of things ($p=0.037$), in calculating ($p=0.009$), in following instructions ($p=0.004$), in solving problems ($p=0.036$), in following directions ($p=0.044$), in performing tasks with the right hand ($p=0.010$) and with vision ($p=0.019$).

In addition, in the case of the questionnaires completed by a close relative the 'exposed' group were reported to have significantly more difficulty in four out of 31 areas relating to language, communication, cognitive and intellectual function and use of hands (data not given), and also in areas relating to personality/mood, namely: depression ($p=0.005$), irritability ($p=0.001$), social withdrawal ($p=0.040$) and confusion ($p=0.036$).

Organophosphates **Critique**

Adequacy of control group

The controls were carefully matched to the cases on an individual basis but potentially confounding differences in verbal intelligence cannot be ruled out. They were obtained from a variety of sources, including referrals from study participants, employee rosters provided by businesses and public agencies, investigator solicitation, and miscellaneous sources. The method by which they were selected from these sources is not described, but it seems likely that many of them were effectively volunteers. It would therefore be expected that there was considerable selection in favour of healthy, public-spirited individuals.

Representativeness of sample

Starting from rosters of poisoning cases, extensive efforts were made to locate as many as possible. Of the 200 individuals who were located and who satisfied the study criteria, a total of 27 refused to participate. Thus the response rate was satisfactory. The response rate for the control group is not given.

Exposure assessment

For the acute poisoning cases, care was taken to ensure that there was a documented history of at least one acute OP poisoning episode, a physician's diagnosis of symptoms consistent with OP poisoning, and no indication of any OP poisonings during the three months prior to the study.

Health outcomes

A large number of neurological tests were carried out with no allowance for multiple testing in the assessment of significance. Therefore, the possibility that one or more of the three significant differences reported is a chance finding must be borne in mind. For the neuropsychological evaluation around half the outcomes were significantly different between the two groups when examined individually, and the overall difference between the two groups was confirmed using a multivariate analysis of variance technique which considered all 34 outcomes simultaneously.

Control of potential confounders

Individual matching for sex, age, socioeconomic status, race and ethnic background should have resulted in good control of confounding.

Interpretation

This was a carefully executed study. It is possible that people with mild neurological deficits are more likely to be accidentally poisoned by pesticides. Also the differences between the poisoned and control subjects may have resulted in part from incomplete matching of verbal intelligence. However, the findings suggest strongly that there are long-term neuropsychological sequelae following acute OP poisoning.

Steenland K, Jenkins B, Ames RG, O'Malley M, Chrislip D, Russo J. Chronic neurological sequelae to organophosphate poisoning. Am J Publ Health 1994; 84: 731-736

This was a study to investigate whether there were any chronic neurological sequelae in subjects who had experienced acute OP pesticide poisoning in California.

Study population

The 'exposed' group consisted of males aged 16 years or more who had suffered from systemic OP pesticide poisoning reported by physicians over the period 1982 to 1990 in California (excluding suicide attempts). A total of 418 potential participants were identified but 56% could not be contacted and 13% refused. Thus the final number was 128 (31%). These were divided into definite cases (83) who reported symptoms consistent with OP poisoning and had a reduction in erythrocyte or plasma cholinesterase activity of at least 20% and the remainder (45) who were considered to be probable cases. No data were available on cholinesterase activities in the latter group but they reported symptoms compatible with toxicity and exhibited relatively specific physical signs (e.g. miosis or bradycardia) or similar symptoms plus a history of direct exposure to OP pesticides of the skin or eye during an application or spill. Virtually all exposures were occupational. A total of 36 involved admission to hospital.

The control group (n = 90) consisted of friends of the poisoned subjects who were not currently working with pesticides. The mean age of the exposed group was slightly higher than that of the control group (33.8 *versus* 29.5 years), but the groups were comparable with regards to race (56% Hispanic), educational attainment and hours of sleep prior to testing. There were more current smokers in the exposed group (37% *versus* 33%) and fewer current drinkers (66% *versus* 72%).

The time period between poisoning and the neurological examination was not reported but the paper was submitted in 1993 and it was stated that the average year of poisoning was 1986, thus, in most cases, several years had passed since the incident.

Exposure

The exposed group had virtually all been involved in occupational exposure to pesticides resulting in an OP poisoning incident reported by a physician. A range of OP pesticides were involved. The primary pesticides suspected of causing the poisoning were as follows. phosalone (20), mevinphos (13), diazinon (11), chloropyrifos (10), dimethioate (7), parathion (5), demeton methyl/oxydemeton methyl (3), others (19), undetermined (40).

Neurological examination

A range of neurological tests were carried out on each subject. Peripheral neuropathy was investigated by three nerve conduction measurements in the dominant arm (median motor, median sensory and ulnar sensory) and two in the dominant leg (peroneal motor and sural sensory). Both conduction velocity and peak amplitude were measured. In addition,

sensitivity to vibration was tested as a measure of possible axonal degeneration in the sensory nerves of the index finger and big toe.

Eight computerised neuropsychological tests from the Neurobehavioural Evaluation System (NES 2) were conducted with instructions in English or Spanish. These covered mood scales; finger tapping (motor speed test); sustained visual attention (continuous performance test); hand-eye coordination (visuomotor accuracy test); symbol digit (coding speed test); pattern memory (visual memory test); and serial digit learning (learning/memory test). In addition the Santa Ana dexterity test and a pursuit aiming test were employed, plus a computerised measurement of postural sway (30 seconds) with eyes open and closed.

Finally, a physician conducted a standard neurological physical examination designed to detect gross neurological abnormalities and obtained a past medical history.

Since an appreciable number of exposed subjects (30%) did not bring a friend to serve as a control, data were analysed without reference to pair matching to facilitate multivariate analysis to control for confounding.

Results

The OP 'exposed' group, after correction for confounding, performed significantly worse on a test for sustained visual attention (continuous performance $p=0.05$) and on two mood scales (increased tension $p=0.02$, increased confusion $p=0.01$). When analysis was restricted to the 'definitely' poisoned group ($n = 83$) or hospitalised cases ($n = 36$) they also showed significantly worse vibrotactile sensitivity of finger and toe ($p<0.01$ for the hospitalised cases, $p=0.03$ to 0.05 for the definite cases). Significant trends of increased impairment were found in those who took more days off work following their poisoning incident over a wide spectrum of tests of both CNS and peripheral nervous system (PNS) function.

Critique

Adequacy of control group

As the control group consisted of friends of the poisoned subjects, it may have been biased in favour of healthy individuals with outgoing personalities who had the time available to take part in the study. Thus, it may not have been representative of the population from which the subjects were drawn, although it is not clear in what direction any resultant biases would have been.

Representativeness of sample

Only 31% of the target population of poisoned subjects were included in the study. There is thus some possibility that they may not have been representative of the whole group.

Exposure assessment

Documented exposures recorded shortly after the poisoning event were available for the poisoned group.

Health outcomes

Twenty seven outcomes were examined. When the outcomes for all the poisoned subjects were compared with those of the control group, three differed significantly at the 5% level. When the length of time taken off work was considered as the explanatory variable (taken as zero for controls) this increased to seven significant differences at the 5% level. For both analyses, one or two significant differences would be expected from chance alone, assuming that the 27 outcomes were independent of each other.

Control of potential confounders

Careful attention was paid to the control of confounding in this study.

Interpretation

This study did not find apparent symptomatic damage to the neurological function of men poisoned in the past but, on the basis of neurological tests, found suggestive evidence of damage to the peripheral nervous system and impairment of central nervous system function.

Organophosphates

Stephens R, Spurgeon A, Calvert IA, Beach J, Levy LS, Berry H, Harrington JM. Neuropsychological effects of long-term exposure to organophosphates in sheep dip. Lancet 1995; 345: 1135-1139

This was a cross-sectional study to compare neuropsychological performance in sheep farmers (with a history of long-term exposure to OP-based sheep dips) with that of non-exposed quarry workers.

Study population

The sheep farmers were recruited by selecting every tenth name on registration lists obtained from the Wool Marketing Board for Devon, Cumbria and North Wales. Farmers were initially contacted by letter. However, this approach produced a low response rate (33%) and a further sample of farmers was recruited by telephone using the same selection procedure with a response rate of 69%. Details of the total eligible population were not given in the paper. Subjects were excluded if they had any nervous system disease or had suffered a head injury resulting in loss of consciousness for longer than 1 hour. Application of these criteria resulted in the exclusion of 52 farmers. A total of 146 male sheep farmers took part in the study. The farmers had not been involved in dipping for two months prior to the study but had previously been exposed to OPs in the course of dipping sheep.

Quarry workers situated in the same three geographical areas were recruited as controls. The response rate overall for the controls was 35%. One hundred and forty three male quarry workers took part in the study. Again, the total eligible population was not stated. Twenty nine quarry workers were excluded because of nervous system disease or previous head injury (defined as for the farmers) or because they had been regularly exposed to pesticides.

There were a number of statistically significant differences in background covariates between the sheep farmers and the controls. These included age (mean age ± SD of 46.8 ± 9.6 years *versus* 40.9 ± 10.8 years), lack of computer familiarity (62% *versus* 43%), and English as first language (77% *versus* 88%).

Exposure

The exposed group comprised farmers involved at some time in sheep-dipping. It was stated that exposure to the OPs used was mainly from dip solution splashing onto the skin, and that protective clothing was seldom worn. An estimate of the extent of exposure was obtained from consideration of a questionnaire covering information on number of sheep, number of years using OPs and number of dips carried out per year. A surrogate measure of dose was obtained from the average number of sheep in the flock, and the number of dips per year and the number of years of using OPs.

The absence of recent exposure was confirmed by analysis of OP metabolites (dialkyphosphates) in urine samples from the sheep farmers which showed concentrations of these metabolites to be in the range observed for unexposed individuals.

Cognitive tests and psychiatric status

A range of tests assessing cognitive function were performed on exposed and control subjects. These included tests for short-term memory (the digit span test, visual spatial memory), sustained attention (the simple reaction time), information processing speed (the symbol-digit substitution test, the syntactic reasoning test) and long-term memory function (category search, the serial word learning test). Subjects were also asked to complete a subjective memory questionnaire and a general health questionnaire to assess psychiatric disorders.

Farmers were tested at home and the quarry workers were tested at their workplace.

There were significant differences in the time of day that the two groups were tested.

Results

The pattern of performance in the two groups was similar in each of the cognitive tests. However, the speed of the performance in the sheep farmers was significantly slower than that of the controls in three of the tests: simple reaction time, symbol-digit substitution and syntactic reasoning. In the simple reaction time test the overall mean reaction times in milliseconds for the farmers were 376 (SD 52.7) compared to 353 (SD 37.4) for the controls ($p<0.0001$). Reaction times towards the end of the test remained statistically significantly slower in the farmers when adjustment was made for covariates ($p<0.05$). In the symbol digit substitution test the mean performance of the farmers was significantly slower than that of controls. After adjustments for covariates, the mean difference in response time between the groups was 1.42 seconds ($p=0.04$). In the syntactic reasoning test farmers were significantly slower than controls for all statement types but had greater difficulty with the more complex negative statements. Farmers were on average 2.3 seconds slower than controls over all levels of statement complexity ($p=0.04$).

In terms of the exposure-response, a significant relation was observed for the syntactic reasoning test ($p<0.0001$) only.

The adjusted means scored from the subjective memory questionnaire did not differ significantly between the two groups ($p=0.39$). From the information reported in the General Health Questionnaire an odds ratio (OR) of 1.5 (95%CI 1.31-1.69; $p=0.035$) was calculated for vulnerability to psychiatric disorders in farmers as compared with controls.

Critique

Adequacy of control group

The authors commented that finding a suitable control group for farmers is difficult. They obviously considered this problem carefully before selecting quarry workers as controls but clearly some doubt must remain as to whether the two groups would have had comparable performances in the absence of OP exposure. The exposed and control groups differed markedly in a number of important characteristics. Although the analysis took some of these factors into account by the use of multiple regression analysis, it is not possible to be

completely sure that the resulting adjustments fully accounted for all relevant differences between the two groups.

Representativeness of samples

The study was one of current workers only. Thus former farmers or quarrymen who had changed jobs or retired early because of their health, or developed symptoms after retirement at the usual age were not represented in the sample. In addition, the low response rate in both farmers and controls must raise concerns that neither group was fully representative of the working populations from which they were drawn. In particular it is possible that farmers who thought that they had been affected by OPs were more willing to participate.

Exposure assessment

Care was taken to carry out the study assessment at a time when farmers had not been involved in dipping for two months, thus eliminating any transient effects of recent exposure, and also to question controls about regular exposure to pesticides. In addition, urine samples were taken from farmers and a sample of controls to confirm that exposure had not taken place within the last 48 hours. The surrogate measure of dose clearly could not take into account differences between farmers in the care they took to avoid exposure or the extent of protective measures used and inaccuracies in this measure would tend to reduce the magnitude of exposure-response found in the analysis. No attempt was made in the analysis to distinguish between different types of OP.

Health outcomes

The results of 16 tests of significance were reported, and differences that were statistically significant at the 5% level were found for five of them. No formal attempt was made to take into account the fact that 16 tests had been carried out, and about one significant result would have been expected by chance alone.

Control of potential confounders

Careful attention was paid to the control of confounding in this study, but as indicated above, it may not have been complete.

Interpretation

The authors point out that there are many reasons why sheep farmers might be more vulnerable to psychiatric disorder than controls. They draw attention to the fact that among the cognitive tests, all the significant differences were for the tests concerned with reaction time and reasoning and that no significant differences were found for the six tests concerned with memory and learning. This specificity makes it less likely that differences were due to major differences between the farmers and controls that were unrelated to OP exposure although the possibility cannot be ruled out. The authors state that the differences found were subtle and unlikely to lead to clinical symptoms.

Stephens R, Spurgeon A, Berry H. Organophosphates: the relationship between chronic and acute exposure effects. Neurotoxicol Teratol 1996; 18: 449-453

This study investigated the relationship between chronic neuropsychological abnormalities and acute exposure effects of OPs in sheep-dippers.

Study population

The exposed population consisted of sheep dippers with long-term exposure to OPs recruited by selecting every tenth name on registration lists obtained from the Wool Marketing Board. Initial selection was by letter but the response rate was only 33%. This was followed by telephone contact resulting in a 69% response. A total of 77 farmers were included in this analysis; these were a subset of the 146 sheep farmers identified in an earlier publication (Stephens *et al.* 1995, also summarised in this section) for whom data on acute effects were available. Exclusion criteria were nervous system disease, head injury with loss of consciousness for an hour or longer, current or prior alcohol-related problems, and recent exposure to other neurotoxic agents.

Exposure to pesticides

The three OPs used in sheep dips at the time of the study were diazinon, chlorfenvinphos or propetamphos. The extent of recent exposure was evaluated by measuring urinary levels of metabolites of diazinon and chlorfenvinphos, namely diethylphosphate (DEP) and diethylthiophosphate (DETP) (no data were available on exposure to propetamphos). It was stated that 43 of the 77 dippers used a dip containing an OP that was detectable by such urinanalysis. Mean combined DEP and DETP excretion for this group the morning following exposure was 44.9 nmoles/mmole creatine (95% CI 26.5 to 63.6). This compared with control levels of 4.7 nmoles/mmole creatine (95% CI 1.0 to 8.3; n=22) and was considered to confirm recent exposure.

Neurological tests

Acute effects were assessed prospectively with measures taken before (baseline) and 24 hours after exposure during each subject's first dipping episode in 1993. A specially designed symptom questionnaire was used for this purpose.

Chronic effects were assessed from a cross-sectional neuropsychological study which was carried out at least two months after any exposure, using computerised neuropsychological tests and questionnaires. Neuropsychological tests were taken from the Neurobehavioural Evaluation System (NES) and the Automated Cognitive Testing System (ACT) and were selected to assess performance over a range of cognitive processes reportedly affected by OP exposure. Also included were the general health questionnaire (GHQ, to assess symptoms of mental ill health) and the subjective memory questionnaire.

Data from these investigations to assess chronic effects were collected in the first half of 1994.

Organophosphates

Results

Simple correlation and multiple linear regression analysis (adjusted for confounders) were used to assess relationships between the change in total symptom reporting from baseline to 24 hours after exposure (i.e. acute effects) and the findings of tests for chronic abnormalities.

There was no evidence of any association between reported acute symptoms and chronic neuropsychological abnormalities.

Critique

Adequacy of control group

This analysis did not rely on any unexposed control group.

Representativeness of samples

The sample of farmers had the same problem of a low response rate as was noted for Stephens *et al.* 1995 (summarised above in this section). As acute effect data were only available for a sub-sample, it would be helpful to know if the chronic effects differed between this group and those for whom these data were lacking; the comparison was only shown for sociodemographic and other background factors. This could be important if recruitment into this sub-sample involved some self-selection and/or selection on health-related criteria.

Exposure assessment

Assessment of short-term exposure for the acute effects was not possible for the 34 of the 77 who used propetamphos.

Health outcomes

Findings were presented in terms of associations between acute and chronic measurements for each of the endpoints in turn. However, only three of these were found to be related to exposure in the previous report. It would be informative to know whether any of these three chronic effects were predicted by other effects immediately following exposure.

Control of potential confounders

Adjustment for confounding appears to have been adequate.

Interpretation

The authors concluded that chronic effects could be present without the prior occurrence of (the same) acute effect. The relevance of the findings depends on the extent to which the subtle chronic abnormalities observed were caused by OP exposure.

Stokes L, Stark A, Marshall E, Narang A. Neurotoxicity among pesticide applicators exposed to organophosphates. Occup Environ Med 1995; 52: 648-653

This study was designed to investigate both acute effects and chronic effects on function of the peripheral nervous system in licensed pesticide applicators in New York State.

Study population

Licensed pesticide applicators were selected from the New York state roster; they were eligible if they were male, held a current pesticide applicator license, were scheduled to spray in the next growing season and were located in the 11 south-eastern counties of the state. A total of 554 were identified, of whom 90 (16%) agreed to participate. For each of these, five potential controls matched for sex, age and county of residence were identified from the Department of Motor Vehicle Records; these were contacted in turn by phone until one agreed to participate.

Exposure to pesticides

It was stated that the most frequently used OP pesticide was azinphos-methyl, which was sprayed on average five or more times a season by 61% of the applicators. Exposure to this OP was verified by measuring urinary concentrations of its metabolite dimethylthiophosphate (DMTP). However, at least ten other OPs were used, and also a range of other insecticides including carbamates, pyrethroids and fungicides. Long-term exposure was estimated from a questionnaire.

It was stated that some protective clothing was worn by most pesticide applicators; 93% used some head covering at least 50% of the time, 60% used overalls and gloves at least 50% of the time. The corresponding proportions for respirators and boots were 35% in each case. About 35% applied pesticides from a tractor cab.

Neurological tests

Acute symptoms were identified from answers to a questionnaire both in the off-season (November to February) and during the spraying season (April to August). Chronic peripheral nerve damage was assessed by measuring vibration sensitivity thresholds in the right and left index fingers and big toes during the off-season.

Results

Regarding acute effects, the only symptom reported in the pesticide applicators significantly more often on-season than off-season was headache (22.2% *versus* 8.8%, OR 6.0, 90%CI 1.01-77.6).

Regarding the chronic effects, paired t tests showed a higher mean vibration threshold score for both dominant and non-dominant ($p<0.04$) hands in the pesticide applicators compared to the controls, indicating impaired performance. No significant effects were found in the feet.

Organophosphates **Critique**

Adequacy of control group

Although the controls were matched to the exposed group for age, sex and county of residence, they were found to have higher socioeconomic status, a higher level of education, and higher alcohol consumption, cigarette consumption, and frequency of neurological disorders, injuries to arms or legs, weakness tingling or numbness in the arms or legs, alcoholism, and batchelor status than the exposed workers. No attempt was made in the analysis to adjust for, or to examine the sensitivity of the results for vibration threshold sensitivity to these differences. Thus some doubt must remain about the comparability of the two groups. The current jobs held by the controls were not described.

For the assessment of acute symptoms and signs, using the measurements made on the same workers during the off-season as a control should provide a highly appropriate comparison.

Representativeness of samples

The study was of current workers only. Those who had changed jobs or retired, perhaps because of their health, were not represented. Response rates were low for both exposed and control groups (16% and 15% respectively), raising concerns that neither group may have been fully representative of the populations from which they were drawn.

Exposure assessment

During the spraying season, three quantitative measures of exposure during the last four days were constructed and were shown to correlate well with levels of DMTP in urine. However, no quantitative measures of exposure were used in the analysis. Based on questionnaire data, exposure was primarily to azinphos-methyl. No information is given as to whether any of those exposed had previously been acutely poisoned.

Health outcomes

The text makes it clear that there was no selection of positive or negative results for presentation, and that all comparisons for which data were collected were published. A total of 26 comparisons were made, of which three were significant at the 5% level, with pesticide applicators either experiencing decreased performance compared with controls or experiencing more symptoms during the spraying season than off season. No corrections were made for multiple testing and so, if there were truly no differences between the two groups, about one significant difference would have been expected by chance.

Control for potential confounders

Careful matching of the controls to the pesticide applicators was carried out in this study.

Interpretation

This study provides fairly clear evidence that, with the possible exception of headaches, the exposures received by the pesticide applicators in this study were not sufficient to induce the 22 symptoms examined as acute effects. It may be of note that the applicators

in this study made quite extensive use of protective measures. The difference between the pesticide applicators and the population controls in vibration threshold sensitivity in the hand may have been a long-term effect of OP pesticide exposure, but could also have been due to exposure to other chemicals among the applicators, or to overall differences in other variables between the two groups. One possible reason for this finding is differences in height, which has been shown to affect vibration threshold sensitivity in other studies, but for which data were not recorded. No significant reduction in vibration threshold sensitivity in the feet of pesticide applicators was observed, but mean thresholds in the feet of the pesticide applicators tended to be lower than in the controls.

Organophosphates

Stoller A, Krupinski J, Christophers AJ, Blanks GK. Organophosphorus insecticides and major mental illness. An epidemiological investigation. Lancet 1965; I: 1387-1388

This study was designed to determine whether areas of Victoria, Australia in which OP pesticide usage was greatest had a higher incidence of mental illness than non-metropolitan areas where there was low pesticide usage.

Study population

All male first admissions, excluding outpatient attendances, to mental health institutes of the Victoria Mental Health Authority were considered. Data on diagnosis, addresses and occupations were abstracted. The Melbourne metropolitan area was excluded from the study but other urban non-metropolitan areas were included. The rural areas included the commercial fruit-growing regions of Victoria.

Exposure

No objective measures of exposure were made. A surrogate measure of exposure was constructed by obtaining information on the total sales of OP pesticides in different regions of Victoria from companies which were responsible for over 90% of the total sales. The sales were related both to area and to number of fruit growers in that region. No information was given as to the identity of the OPs.

Measures of mental illness

The hospital admissions were classified as schizophrenic states, depressive states (psychotic and neurotic), other psychoses and deferred diagnosis. These were chosen to represent illnesses which might be connected with OP exposure. Conditions which could be considered to be unrelated, i.e. alcoholic psychoses, senile brain disorders and mental deficiency, were excluded.

Results

The regions were categorised as urban non-metropolitan or rural; the two regions, Goulburn Valley and Murray Valley, with the greatest acreage of fruit growing, numbers of growers, and sales of OP pesticides were also considered separately. The total incidence of admissions for mental illness per 100,000 males in these two regions was not greater than in the urban non-metropolitan regions nor in rural areas. There was also no significant difference in the incidence specifically of admissions for schizophrenic states or depression. The incidence of schizophrenia, in Goulburn Valley was significantly greater than in Murray Valley (29.0 per 100,000, n=18 *versus* 14.7 per 100,000, n=3 respectively). However, none of the 18 individuals diagnosed as schizophrenic in Goulburn Valley had been exposed to OP pesticides nor they had worked on a fruit farm.

Critique

Adequacy of control group

The comparison between different areas of Victoria appears valid.

Representativeness of sample

The entire population of each area was considered and no sampling was involved.

Exposure assessment

The measures appear valid.

Health outcomes

Admissions to psychiatric hospitals for particular diagnoses would seem to be a valid measure and not subject to bias.

Control for potential confounders

It appears that no adjustment for age was carried out in the analysis of this study and so some confounding by age may have occurred.

Interpretation

In interpreting this study it should be remembered that it is a geographical study comparing disease incidence in different areas according to the average characteristics of these areas, rather than a study of individuals. However, it shows clearly that OP exposure did not cause a higher rate of hospital admissions for schizophrenic and depressive states in the area with highest sales of insecticides per acre and per grower.

Organophosphates

Reasons why other epidemiological studies were not considered as relevant as the 'key' studies summarised in detail

Aden-Abdi Y, Villén T, Ericsson Ö, Gustafsson LL, Dahl-Puustinen M-L. Metrifonate in healthy volunteers: interrelationship between pharmacokinetic properties, cholinesterase inhibition and side-effects. Bull World Health Org 1990; 68: 731-736

This paper describes a trial of metrifonate in healthy volunteers. The design appears sound but information is provided only about acute side effects.

Beach JR, Spurgeon A, Stephens R, Heafield T, Calvert IA, Levy LS, Harrington JM. Abnormalities on neurological examination among sheep farmers exposed to organophosphorous pesticides. Occup Environ Med 1996; 53: 520-525

This paper describes findings on standardised neurological examination of a subset of subjects from the study summarised above of Stephens et al., 1995. The study design is inappropriate in that it compares symptomatic, exposed subjects with asymptomatic, unexposed subjects. Other limitations are the small sample size, the fact that examinations were not fully blinded and doubts about the validity of the two point discrimination tests employed.

Behan PO. Chronic fatigue syndrome as a delayed reaction to chronic low-dose organophosphate exposure. J Nutr Environ Med 1996; 6: 341-350

This paper compares symptomatic, exposed subjects with asymptomatic, unexposed controls – an unsatisfactory study design.

Burns CJ, Cartmill JB, Powers BS, Lee MK. Update of the morbidity experience of employees potentially exposed to chlorpyrifos. Occup Environ Med 1998; 55: 65-70

This paper reports on health outcomes among employees potentially exposed to chlorpyrifos in its manufacture. The assessment of outcomes was based only on company medical records, and there was no follow-up of leavers. Thus, the ability of the study to detect adverse effects was limited.

Davies JE. Neurotoxic concerns of human pesticide exposure. Am J Ind Med 1990; 18: 327-331

This is a review article and contains no original data.

Drenth HJ, Ensberg IFG, Roberts DV, Wilson A. Neuromuscular function in agricultural workers using pesticides. Arch Environ Health 1972; 25: 395-398

This paper describes EMG findings in 102 male agricultural workers. The selection of subjects and response rates are not well described. Furthermore, there was no internal control group, and it is unclear how repeatable the EMG test is in

unexposed people and whether the normal values employed were appropriate. Nor was any separate analysis presented for OP exposure specifically.

Durham WF, Wolfe HR, Quinby GE. Organophosphorus insecticides and mental alertness. Arch Environ Health 1965; 10: 55-66

This study involved tests of mental alertness in people with recent exposure to OPs and controls with no exposure. No information is available about the long-term exposure of the subjects, and it is unclear exactly how the controls were selected. The possibility of confounding by factors other than OP exposure cannot be satisfactorily excluded.

Fleming LE, Bean JA, Rudolph M, Hamilton R. Mortality in a cohort of licensed pesticide applicators in Florida. Occup Environ Med 1999; 56: 14-21

This paper describes a retrospective cohort study of mortality amongst licensed pesticide applicators in Florida. No data were available on the specific pesticides to which subjects had been exposed, or whether these included OPs.

Horowitz SH, Stark A, Marshall E, Mauer MP. A multi-modality assessment of peripheral nerve function in organophosphate-pesticide applicators. J Occup Med 1999; 41: 405-408

This study assessed peripheral nerve function in nine pesticide applicators from an earlier investigation summarised above (Stokes et al., 1995) with relatively high exposures and sensory thresholds for vibration. Because of its limited size, the way in which subjects were selected, and the uncertain validity of the normative data that were used, only limited conclusions can be drawn.

Jusic A, Jurenic D, Milic S. Electromyographical neuromuscular synapse testing and neurological findings in workers exposed to organophosphorous pesticides. Arch Environ Health 1980; 35: 168-175

This paper compares results of electromyographic neuromuscular synapse testing and neurological findings in workers before and after exposure to OPs. As such, it does not provide information about long-term toxicity.

Korsak RJ, Sato MM. Effects of chronic organophosphate pesticide exposure on the central nervous system. Clin Toxicol 1977; 11: 83-95

This study compared a neuropsychological test and computer-analysed EEGs in subjects with different levels of chronic exposure to OPs. The design of the study is poorly described, and the origin of the subjects not properly reported. It is unclear whether or not some of the subjects had recent exposure to OPs. Also, differences in age and other potential confounders may not have been adequately controlled.

Organophosphates **Levin HS, Rodnitzky RL, Mick DL. Anxiety associated with exposure to organophosphate compounds. Arch Gen Psychiatry 1976; 33: 225-228**

This study compared psychiatric morbidity in 13 commercial pesticide applicators and 11 farmers with 24 matched controls. It is unclear whether the controls, who were farmers, were appropriate for the subset of commercial sprayer exposed subjects. No information is provided about possible poisoning episodes in the past, and some of the controls may have been exposed to OPs previously. There was a potential for residual confounding by differences in alcohol use. The small size of the study meant that it had low statistical power.

London L, Myers JE. Use of a crop and job specific exposure matrix for retrospective assessment of long term exposure in studies of chronic neurotoxic effects of agrichemicals. Occup Environ Med 1998; 55: 194-201

This paper describes a job-exposure matrix used as part of a study to assess the neurotoxicity of long-term exposure to agricultural chemicals. It provides useful background information in relation to several other reports from the same study that the Working Group considered in more detail. However, it does not give any new information about the health risks from OPs.

Metcalf DR, Holmes JH. EEG, psychological, and neurological alterations in humans with organophosphorus exposure. Ann N Y Acad Sci 1969; 160: 357-365

This paper reviews a programme of research, but provides only a limited description. It offers no clear information regarding the long-term toxicity of low-level exposure to OPs.

Parrón T, Hernández AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. Hum Exp Toxicol 1996; 15: 957-963

This paper describes clinical findings and biochemical results in greenhouse sprayers of pesticides. The subjects were all volunteers and it is unclear whether they were representative. Furthermore, they had exposure to multiple pesticides and the proportion specifically exposed to OPs is unclear. Some may have had recent exposures. People who had been out of work recently with illness were excluded, limiting the ability of the study to detect more serious health effects. The comparisons presented were between people with high and low exposure, and no unexposed control group was included. There was potential for recall bias, and control for confounders such as age and type of job may not have been adequate. Multiple statistical tests were carried out, and the *a priori* suspicion regarding some of the associations tested was relatively weak.

Parrón T, Hernández AF, Villanueva E. Increased risk of suicide with exposure to pesticides in an intensive agricultural area. A 12-year retrospective study. Forensic Sci Int 1996; 79: 53-63

This paper describes geographical differences in suicide rates which the authors suggest may be related to OP exposure. The comparisons were not age-standardised, and no data are provided on the risk of suicide by occupation or by OP exposure.

Ramos OD, Almirall P, Sánchez R. Evaluacion de funciones psicomotoras en trabajadores expuestos habitualmente a plaguicidas [Evaluation of psychomotor functions in workers exposed habitually to pesticides]. Rev Cub Hig Epidemiol 1986; 24: 103-110

This paper describes psychomotor function in a sample of workers exposed to pesticides. The exposed group may not all have worked with OPs, and it is unclear how long had elapsed since their last exposure. The issue of confounding is not adequately addressed.

Rayner MD, Popper JS, Carvalho EW, Hurov R. Hyporeflexia in workers chronically exposed to organophosphate insecticides. Res Comm Chem Pathol Pharmacol 1972; 4: 595-606

This is a preliminary report describing hyporeflexia in agricultural workers exposed primarily to OPs. The exposed subjects were all known to be in good general health, limiting the ability of the study to detect more serious health effects. Moreover, there was a possibility that they had been exposed within the last few days. The source of controls and response rates are unclear, as are the statistical methods.

Richter ED, Chuwers P, Levy Y, Gordon M, Grauer F, Marzouk J, Levy S, Barron S, Gruener N. Health effects from exposure to organophosphate pesticides in workers and residents in Israel. Isr J Med Sci 1992; 28: 584-598

This paper summarises a programme of research, but individual studies are not fully described. They mostly relate to acute health effects.

Roberts DV. E.M.G. voltage and motor nerve conduction velocity in organophosphorus pesticide factory workers. Int Arch Occup Environ Health 1976; 36: 267-274

This paper describes several studies of EMGs in factory workers exposed to OPs. The investigations are poorly documented and confounding effects cannot be satisfactorily excluded. At the same time, more serious effects may have been missed because of healthy worker selection.

Rodnitzky RL, Levin HS, Mick DL. Occupational exposure to organophosphate pesticides. A neurobehavioral study. Arch Environ Health 1975; 30: 98-103

This study compared neuropsychological tests in 23 subjects exposed to OPs and an unexposed control group. It has a number of limitations including its low statistical

power, inadequate information about the selection of subjects and response rates, doubts about the appropriateness of using farmers as controls for commercial pesticide applicators, the fact that some of the controls may have been exposed to OPs in their work, the fact that exposed subjects had recent exposure, and the possibility of uncontrolled confounding.

Rosenstock L, Daniell W, Barnhart S, Schwartz D, Demers PA. Chronic neuropsychological sequelae of occupational exposure to organophosphate insecticides. Am J Ind Med 1990; 18: 321-325

This is only a preliminary report and does not present any results.

Sack D, Linz D, Shukla R, Rice C, Bhattacharya A, Suskind R. Health status of pesticide applicators: postural stability assessments. J Occup Med 1993; 35: 1196-1202

This paper describes tests of postural sway in 37 pesticide-exposed workers and 35 unexposed subjects. The subjects were all volunteers, and more serious health effects may have been missed because of healthy worker selection. It is unclear to what extent subjects had recently been exposed to OPs. The controls came from the University of Cincinnati community and differed educationally and socioeconomically. As such they may not have been appropriate. There is a possibility of residual confounding by alcohol and caffeine.

Simkin S, Hawton K, Fagg J, Malmberg A. Stress in farmers: a survey of farmers in England and Wales. Occup Environ Med 1998; 55: 729-734

This study does not provide direct information on the risk of health effects in relation to OPs, although it does give useful data on the prevalence of symptoms among farmers as a whole.

Stålberg E, Hilton-Brown P, Kolmodin-Hedman B, Holmstedt B, Augustinsson K-B. Effect of occupational exposure to organophosphorus insecticides on neuromuscular function. Scand J Work Environ Health 1978; 4: 255-261

This study of neurophysiological testing in 11 pesticide sprayers exposed to OPs is limited by its low statistical power and its inability to distinguish acute from chronic effects.

Appendix 5
The Institute of Occupational Medicine epidemiological study: summary and critique

General introduction

This Appendix considers the recent IOM study. The Working Group were provided with considerably more detailed information on the study than could be summarised in a published paper. They had a lengthy discussion with the authors of the report who designed and carried out the investigations. The Working Group considered that this study was important to its work. It focused on an exposed population of particular concern, namely sheep dippers in the UK. The Group applied the same criteria for appraisal of this study as for the other epidemiological studies considered.

The work described in the report covered three phases. These are considered in turn. The tables and the figure are adapted from those in the report of the appropriate phase of the study.

Phase I

Sewell C, Pilkington A, Buchanan D, Tannahill SN, Kidd M, Cherrie B, Robertson A. ***Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers. Phase 1. Development and validation of an organophosphate uptake model for sheep dippers*****. Report No. TM/99/02a from the Institute of Occupational Medicine, Edinburgh:Institute of Occupational Medicine, 1999**

Aim

Sheep dipping practices were studied during the summer of 1996 at sites using diazinon-based products in order to develop a model for uptake of OPs; this was validated by comparison with levels of diazinon metabolites in urine.

Design

One-day surveys were carried out on 20 dipping sessions at sheep farms in Scotland, mainly in the Borders. The activities involved in dipping were observed and recorded. The observations were of the frequency and extent of handling the concentrated sheep dip, the extent and time of contact with dip solution itself, the protective clothing worn, hand washing, smoking and eating habits and any significant incidents. Individuals were classified as working in one or more of the following three job categories; 'Helper', the worker who herds the sheep ready to enter into the dipping bath, 'Chucker', the worker

who feeds the sheep into the dipping bath, and 'Paddler', the worker who plunges the sheep into the dip.

Each individual was asked to provide urine samples before dipping, at the end of the day's dipping, early the next morning and 24 hours later. The uptake of OPs was assessed from the increment in levels of the urinary metabolites of diazinon, diethylphosphate (DEP) and diethylphosphorothioate (DEPT). The data were examined in relation to the exposure indices using a combination of scatter plots and multiple linear regression analysis.

Results

Urinary metabolites

An overall summary of the urinary metabolite levels found in the 54 individuals from whom data were available is given in Table A5.1. Pre-dip concentrations were low (median urinary DEP concentration: 5.7 nmoles/mmole creatinine) with 22 out of the 54 individuals (41%) having no detectable DEP or DEPT. Urinary concentrations of DEP and DEPT at both post-dip sampling times were higher than before dipping, with a median increment of urinary DEP+DEPT concentration of 9.0 nmoles/mmole creatinine from pre-dip to the sample collected the next morning.

Table A5.1: Mean concentrations of metabolites DEP and DEPT measured in urine samples (nmoles/mmole creatinine) including the median, standard deviation (SD), minimum and maximum values and the first and third quartiles (Q1, Q3)

Metabolite	Time	n	Mean	Median	SD	Min	Max	Q1	Q2
DEP	Pre-dip	54	5.7	3.4	6.8	0.0	28.4	0.0	10.4
	Post-dip	48	10.6	6.7	15.1	0.0	85.7	0.0	14.3
	Next morning	52	15.0	10.4	16.6	0.0	82.4	5.7	17.8
	Post-dip – Pre-dip	46	5.8	4.5	12.6	-10.6	64.0	0.0	9.4
	Next morning – Pre-dip	50	9.5	7.4	14.8	-28.4	60.7	0.8	13.7
DEPT	Pre-dip	54	5.1	0.0	9.7	0.0	47.1	0.0	6.6
	Post-dip	48	27.7	11.2	55.0	0.0	348.0	1.1	29.3
	Next morning	52	14.3	6.2	22.2	0.0	105.2	0.0	20.0
	Post-dip – Pre-dip	46	23.2	8.4	53.5	-10.3	321.4	0.0	23.5
	Next morning – Pre-dip	50	9.2	1.6	24.7	-32.1	105.2	0.0	14.4
DEP+DEPT	Pre-dip	54	10.8	5.7	14.6	0.0	57.1	0.0	15.8
	Post-dip	48	38.2	14.9	68.2	0.0	433.7	7.8	44.5
	Next morning	52	29.3	19.2	32.0	0.0	127.6	9.5	38.1
	Post-dip – Pre-dip	46	29.0	12.8	63.6	-20.9	385.4	0.0	30.4
	Next morning – Pre-dip	50	18.6	9.0	32.9	-47.4	127.6	0.0	25.7

Sources of exposure

The most important source of exposure to OP was contact with the concentrated dip, which occurred almost always on the hands and usually as a result of handling the concentrate container during the preparation and replenishment of the dipping bath. It was noted that contamination of the hands and the fingers (or gloves when gloves were worn)

occurred almost every time the concentrate container was handled. Levels of urinary metabolites were found to increase with the frequency of handling the concentrate. Simple linear regression analysis of data from the 42 individuals involved in handling the concentrate indicated that it accounted for 56% of the overall variance in uptake (R^2) based on a mean (± SD) of 4.4 ± 0.61 concentrate handling events. If one outlier was omitted, R^2 increased to 64%.

It was noted that the use of gloves and other personal protective equipment produced little benefit with regard to uptake, probably because these were usually in poor condition.

Since the extent of exposure to concentrate was the major determinant of OP uptake and may have masked any weaker effects due to exposure to the dipping solution itself, the effect of exposure to the latter was investigated in a sub-group of 21 individuals who did not handle the concentrate. This was possible because it was usually only a single individual at each farm who was responsible for replenishing the bath with the concentrate. A positive association was seen when urinary metabolite increment was plotted against a time weighted "splash score" in those who did not handle the concentrate. Urinary metabolite levels were on average much lower than in workers exposed to the concentrate.

The extent of splashing with the dip was related to an individual's proximity to the dipping bath. In general, paddlers received the most splashing, in particular to the legs and feet. Lower legs and feet were often recorded as being soaked although splashing usually occurred in all body areas. The extent of splashing on chuckers was more variable, with proximity to the bath being important as well as working practices. Helpers were splashed the least, principally because they worked away from the dipping bath and the source of contamination.

It was noted that a less important source of contamination was contact with the treated fleece or aerosols arising from these sources. When sheep left the bath they were always collected in draining pens where they usually shook their fleece vigorously to remove dip wash. At all sites except one the pens were away from the workers or high-sided screens were used to control this source of exposure. On occasions, however, sheep shook their fleece vigorously to remove excess dip wash on leaving the bath and prior to reaching the pens, resulting in additional contamination of the paddler. Although not all farms had remotely operating gates to the draining pens it was noted that most individuals took care when manually releasing sheep to avoid contact with treated fleece.

The exposure model

On the basis of the data generated the model proposed for the uptake of OPs (UPTAKE) during a full sheep dipping session was as follows:

$$\mathbf{UPTAKE} = \boldsymbol{a} \times \mathbf{CONC} + \boldsymbol{b} \times \mathbf{DIP}$$

The model required inputs from the two main sources of exposure identified in the study, CONC representing concentrate and DIP representing dip wash. CONC represents the

expected number of times concentrate is handled. DIP represents the expected time-weighted splash score if an individual were observed and data recorded in a manner similar to this study. From the regression analysis which jointly fitted terms for concentrate and dip wash, estimates for the coefficients of a and b were 3.6 and 0.2 respectively. It was acknowledged in developing this model that other factors such as interindividual variation and other unconfirmed sources of exposure could have a significant effect on uptake.

Phase 2

Pilkington A, Buchanan D, Jamal GA, Kidd M, Sewell C, Donnan P, Hansen S, Tannahill SN, Robertson A, Hurley JF, Soutar CA. *Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers. Phase 2. Cross-sectional exposure-response study of sheep dippers*. Report No.TM/99/02b from the Institute of Occupational Medicine, Edinburgh: Institute of Occupational Medicine, 1999

Aim

The aim of this study was to investigate the relationship between cumulative exposure to OPs and clinically relevant indices of peripheral neuropathy.

Design

This was a cross-sectional study to compare exposure to sheep dips and chronic peripheral neuropathy.

For practical reasons the study was based on two areas of the UK with a high density of sheep farming. These were Hereford and Worcester in England and the Borders, Lothians and Ayrshire in Scotland. Suitable farms were identified from a sampling frame constructed from annual census data maintained by MAFF or the Scottish Office.

Population studied

A total of 995 sheep farm owners were sent letters of invitation of whom 612 (61%) agreed to participate. For comparative purposes two groups not exposed to sheep dips were used. These comprised 53 farmers with no sheep dipping experience and 107 ceramics workers ('ceramics').

Exposure data

Information on exposure was obtained from the questionnaire developed through phase 1 of the study. This was based on easily identified and relatively stable factors of sheep dipping practice which had been shown in phase 1 to have been related to uptake and

which were considered to be amenable to recall in the phase 2 survey. The main features included were flock size, concentrate handling and principal task/job.

Assessment of neurological symptoms and sensory tests

The neurological assessment was based on the Mayo Clinic methodology but with omission of the full battery of neurophysiological tests which were not appropriate for a field study to be carried out by a non-specialist. It involved a symptom questionnaire in conjunction with a series of quantitative sensory tests (QST). The questionnaire was somewhat modified from that used in the original Mayo Clinic method in order to make it appropriate for use by trained technicians on farms. Two automated QST tests for measuring thermal thresholds (hot and cold) in the skin at the top of the foot (the most distal site of the lower limb that can be tested) were employed. In these tests the individual indicates when he can feel the probe becoming hot (or cold). A test to measure vibration thresholds was also employed, the test site used being the top of the foot in line with the big toe (middle of the first metatarsal bone).

Results

Exposure

The median duration of occupational exposure of the sheep dippers during their lifetime was 54 days dipping but a few farmers had dipped on over 1000 days. Total dipping days were highly correlated (r=0.92) with the model based exposure index. Age at survey was not correlated with any of the exposure indices.

Neurological assessment

QST tests

An unexpectedly high number of the controls who were not exposed to sheep dips, were found to have abnormal sensory thresholds based on a comparison with clinical reference values. This was particularly the case for the cold QST test and, to a lesser extent, the vibration test. For example 48% of the ceramic workers were regarded as positive for an abnormal cold threshold. The anomaly was attributed to a possible sensitivity of the test method to limb temperature, and the related core temperature at the time of testing. Ambient temperatures were low at the time of the field measurements. It was therefore decided that the use of clinical reference values to define abnormalities in thresholds measured in the field could not be justified, and that the symptom score and the three sensory test thresholds should each be analysed separately in relation to exposure.

Age was found to be positively related to all three sensory test thresholds, and on average males had higher thresholds than females. After adjustment for age and sex, there were inconsistent differences between the occupational groups in England and Scotland with regard to hot and vibration thresholds. The cold thresholds were, however, higher in the farmers involved in sheep dipping than in the other groups. On average the values obtained in the sheep farmers were 1.35 times (95% confidence interval, 95%CI, 1.14 to

Organophosphates 1.60) higher than the average for the ceramic workers and 1.65 times (95%CI, 1.31 to 2.07) higher than the average amongst the non-sheep dip farmers.

Symptoms

The crude prevalence of reported symptoms was highest amongst the farmers involved in sheep dipping (19%), followed by that in the other farmers (11%) and then the ceramic workers (5%). The symptoms data are summarised in Table A5.2. Autonomic symptoms were more frequently reported than sensory or motor symptoms.

Table A5.2: Prevalence (%) of reported non-zero symptom scores

	Age (years)				
Symptom group	**<35**	**35–44**	**45–54**	**>=55**	**All**
A) Muscle weakness					
ceramics	0.0	0.0	0.0	0.0	0.0
NSD farmers	4.2	0.0	0.0	0.0	1.9
SD farmers	3.0	7.0	7.4	14.2	7.8
B) Sensory symptoms					
ceramics	0.0	2.5	0.0	14.3	1.9
NSD farmers	0.0	14.3	5.9	20.0	5.7
SD farmers	5.4	11.3	11.4	18.7	11.6
C) Autonomic					
ceramics	17.1	5.0	5.3	14.3	10.3
NSD farmers	25.0	28.6	17.6	0.0	20.8
SD farmers	26.5	24.6	26.8	35.5	28.4
D) Overall					
ceramics	4.9	5.0	0.0	14.3	4.7
NSD farmers	12.5	14.3	5.9	20.0	11.3
SD farmers	9.0	16.9	20.1	31.0	19.1
Total numbers of individuals in each age group (from which prevalence rates were calculated)					
ceramics	41	40	19	7	107
NSD farmers	24	7	17	5	53
SD farmers	166	142	149	155	612

NSD = non-sheep dip
SD = sheep dip

Association of neurological findings with exposure to OPs

Of the four neurological response variables, only symptoms were positively related to cumulative exposure based on the uptake model for phase 1 and after adjustment for important confounders. See Table A5.3 for the data on symptoms and total number of days involved in sheep dipping. However the statistical significance of this relationship depended upon a very small number (four) of farmers with very high exposure.

Table A5.3: Prevalence of reported symptoms by occupational group and number of days dipped

Days	Scotland			England			All		
dipped	N	n	%	N	n	%	N	n	%
Ceramics									
0	36	2	5.6	71	3	4.2	107	5	4.7
Farmers									
0	46	5	10.9	7	1	14.3	53	6	11.3
1-100	218	26	11.9	231	56	24.2	449	82	18.3
101-200	81	15	18.5	25	7	28.0	106	22	20.8
201-400	30	6	20.0	7	3	42.9	37	9	24.3
>400	15	1	6.7	5	3	60.0	20	4	20.0

N = total number
n = number showing any neurological symptom

Further analysis of exposure-effect characteristics showed that the average concentrate handling intensity, independent of duration of exposure, could explain the difference between sheep dip farmers and ceramic workers in relation to both symptom reporting and to a lesser extent cold threshold. The data on symptoms with respect to concentrate handling, country and sex are shown in Table A5.4. Those who had ever acted as principal concentrate handler reported more symptoms than those who had not, the odds ratio (OR) being 3.43 (95%CI, 1.63 to 7.23). In addition there was a trend towards an effect with duration of exposure, which was not statistically significant at the 5% level.

Table A5.4: Odds ratios (OR) and 95% confidence intervals (95%CI) for prevalence of reported symptoms compared to various factors including the effects of sex and concentrate handling

Terms	Model*	
	OR	95%CI
Age		
(x10^{-1} years)	1.43	(1.22 to 1.67)
Country		
(England *versus* Scotland)	1.98	(1.30 to 3.03)
SD farmers *versus* NSD farmers	0.37	(0.12 to 1.15)
SD farmers *versus* Ceramics	1.27	(0.40 to 4.00)
DAYS		
(xIQR^{-1})	1.10	(0.99 to 1.23)
Sex		
(male *versus* female)	0.34	(0.17 to 0.70)
Concentrate handler		
('ever' *versus* 'never')	3.43	(1.63 to 7.23)

IQR = inter-quartile range (74 for DAYS)
SD = sheep dip
NSD = non sheep dip

* The Odds Ratios for the prevalence of symptoms were modelled using linear logistic regression analyses including the terms listed (e.g. Age, Country, etc.).

Organophosphates Adjustment for concentrate handling revealed a significantly lower rate of symptoms reported amongst males compared to females of the same age, country and exposure (OR = 0.34; 90%CI, 0.17 to 0.70).

There was a significantly higher prevalence of symptoms in the English study group as compared to the Scottish group with an OR of 1.98 (95%CI, 1.30 to 3.03). Subsequent comparison of the OP sheep dip products recalled by farmers did not reveal any marked differences in product usage.

Some relation to concentrate handling intensity was seen for all three sensory tests. This was more marked in the case of the cold and vibration thresholds. These data are summarised in Tables A5.5 and A5.6. This association peaked at around the mid-point of the intensity range (four handling events per day), as shown in Figure A5.1.

Table A5.5: Cold QST threshold: estimated multiplicative effects and 95% confidence intervals (95%CI) including the effects of important confounders, cumulative exposure and average concentrate handling intensity

Terms	Model*	
	Multiplicative effect	95%CI
Age		
($x10^{-1}$ years)	1.30	(1.24 to 1.36)
Sex		
(male *versus* female)	1.26	(1.04 to 1.54)
OG (occupational group)		
SD farmers *versus* NSD farmers	1.49	(1.15 to 1.91)
SD farmers *versus* Ceramics	1.21	(0.98 to 1.49)
DAYS		
($xIQR^{-1}$)	0.98	(0.95 to 1.02)
Ave. CONC	3.50	(1.28 to 9.58)
$(Ave. CONC)^2$	0.23	(0.06 to 0.82)

IQR = inter-quartile range (74 for DAYS)
SD = sheep dip
NSD = non sheep dip

Ave. CONC is obtained by dividing the cumulative exposure by the number of days dipped. It is a measure of the average intensity of exposure.

* The logarithm of the threshold was modelled using multiple linear regression analysis including the terms listed (e.g. Age, Sex, etc.). The estimates given have been transformed back to the linear scale and are therefore multiplicative effects.

Table A5.6: Vibration QST threshold: estimated multiplicative effects and 95% confidence intervals (95%CI) including the effects of important confounders, cumulative exposure and average concentrate handling intensity

Terms	Model*	
	Multiplicative effect	95%CI
Age		
(x10^{-1} years)	1.62	(1.53 to 1.71)
Sex		
(male *versus* female)	1.03	(0.81 to 1.31)
Country x Occupational group interaction:		
(Scottish SD farmers versus)		
Sco. NSD farmers	0.86	(0.62 to 1.20)
Sco. Ceramics	1.40	(0.98 to 2.01)
Eng. SD farmers	1.07	(0.91 to 1.25)
Eng. NSD farmers	1.57	(0.76 to 3.27)
Eng. Ceramics	0.84	(0.63 to 1.12)
DAYS		
(x IQR^{-1})	1.00	(0.95 to 1.05)
Ave. CONC	7.41	(2.18 to 25.18)
$(Ave. CONC)^2$	0.10	(0.02 to 0.48)

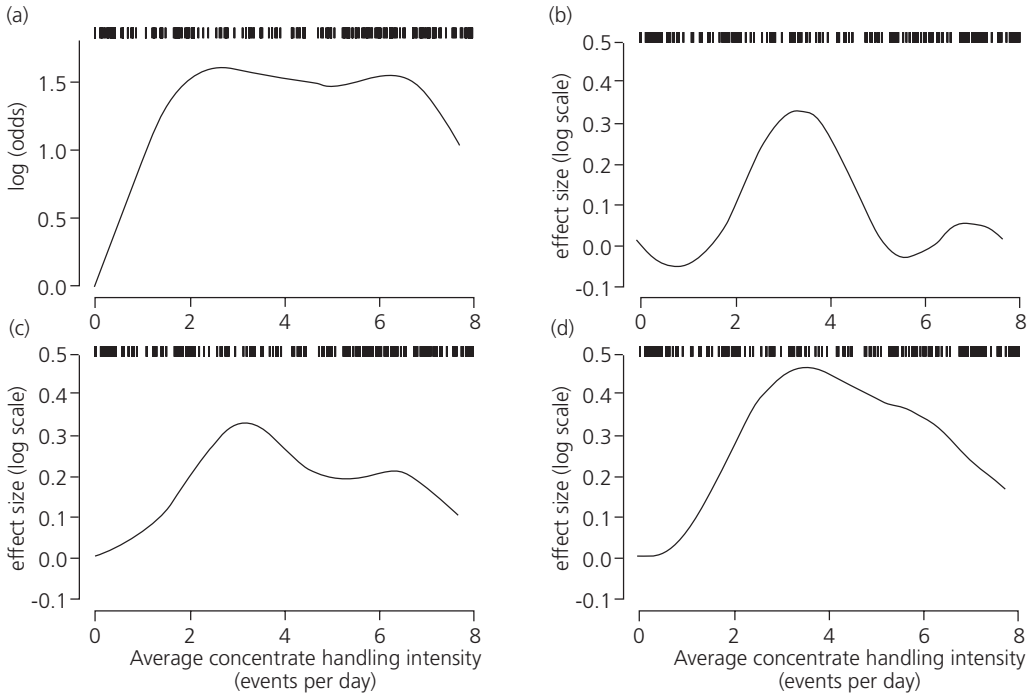
IQR = inter-quartile range (74 for DAYS)
SD = sheep dip
NSD = non sheep dip

Ave. CONC is obtained by dividing the cumulative exposure by the number of days dipped. It is a measure of the average intensity of exposure.

* The logarithm of the threshold was modelled using multiple linear regression analysis including the terms listed (e.g. Age, Sex, etc.). The estimates given have been transformed back to the linear scale and are therefore multiplicative effects.

Organophosphates

Figure A5.1: Effect of average concentrate handling exposure intensity, relative to zero exposure and adjusted for confounders, using a cubic smoothing spline for a) symptoms and b) hot, c) cold and d) vibration thresholds



Explanation of x and y axes

The x axis gives a measure of the average exposure to the concentrate during one day's dipping.

Each time the concentrate is handled this is measured as an exposure. Dippers who were always the principal concentrate handler would have an average exposure of 8, whereas those who never handled concentrate would have an average of zero. Individuals who handled concentrate on some dipping days but not others will have intermediate average values. The "bar code" across the top of each graph is identical for all graphs. There is a line in this bar for each individual at the appropriate average exposure intensity.

The y axis shows, on log scales, (a) the increased probability of reporting symptoms or (b, c, d) the change in sensory threshold (hot, cold, vibration respectively) for individuals at each exposure intensity, taking age, sex and country of origin into account.

Only the data for sheep dippers were used for these graphs. The lines are calculated smooth curves through the individual data points.

Phase 3

Pilkington A, Jamal GA, Gilham R, Hansen S, Buchanan D, Kidd M, Azis MA, Julu PO, Al-Rawas S, Ballantyne JP, Hurley JF, Soutar CA. *Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers. Phase 3. Clinical neurological, neurophysiological and neuropsychological study*. Report No. TM/99/02c from the Institute of Occupational Medicine, Edinburgh:Institute of Occupational Medicine, 1999

Aims

The aim of phase 3 was to classify, in terms of clinical disease, the subjects with indices of peripheral neuropathy identified in the phase 2 field studies; to describe any association between neurological and neuropsychological abnormalities; and to examine evidence for a relationship between neuropsychological status and estimated cumulative OP exposure.

Study design

This was a nested clinical study of selected subjects with and without peripheral neurological abnormalities identified in phase 2. The study was carried out about 18 months after the field studies in phase 2.

Study population

The study population consisted of a subset of 76 individuals (from 79 chosen) from the sheep farmers in phase 2. They were subdivided on the basis of the results obtained in phase 2 into 17 with no neuropathy, 36 with possible neuropathy and all 23 subjects who were considered to show probable/definite neuropathy. This classification depended on a neuropathy scoring system that used information from the symptom questionnaire and QST tests.

Neurological assessment

Each participant was subjected to the same questionnaire as used in phase 2, but in this case it was administered by a neurologist in the clinic. The same QST tests were carried out, but again in the clinic rather than in the field. In addition nerve conduction measurements and electromyography were performed. Each individual was also given a battery of neuropsychological tests to assess general intelligence, psychomotor function, attention, memory, mood and affect. These tests were performed without knowledge of the results from phase 2.

Results

Reproducibility of symptoms and QST measures in the field and clinic

A comparison of the phase 2 (field) and phase 3 (clinic) classification using the neuropathy scoring system indicated that 40 (51%) of the 79 individuals were classified in the same category on both occasions. Overall agreement was only modestly better than chance (kappa statistic, k = 0.26, SE = 0.08). Amongst the 3 QST outcomes, agreement was significantly better than chance for the vibration threshold (k = 0.30) and the hot threshold (k = 0.22) but not for the cold threshold (k = 0.18). It was judged that the adverse conditions in the field measurements (low temperature) were the reason for the low correlation in the latter case. The use of normative data from the clinic for the field study measures was a significant factor in this difference in classification.

Organophosphates There was better agreement between symptoms reported in the field studies and those in the clinic. Complete agreement was found in the case of 51 individuals (65%); this was significantly better than chance (k = 0.37).

Additional neurological tests

The overall results obtained from the neurological/neurophysiological studies are summarised in Table A5.7.

Twenty three (32%) out of the 72 subjects had confirmation of their neuropathy by identification of neurological signs or nerve conduction abnormality. These subjects were stated to have a neuropathy in the clinical sense and would be reported as such to a referring GP or clinician. Of these 23 subjects with peripheral neuropathy 12 had been classified as having definite/probable neuropathy in phase 2 (out of 23), 10 as having possible neuropathy (out of 34) and one as having no neuropathy (out of 15).

Ten (29%) of the 34 individuals classified as having 'possible neuropathy' in phase 2 had confirmed evidence of neuropathy. Three of these had neurological signs and symptoms and/or abnormal QST. One of the three also had an abnormal electromyogram (EMG). The remaining seven showed symptoms/abnormal QST suggestive of neuropathy together with abnormal nerve conduction. A further six subjects with peripheral neuropathy had abnormal EMGs in distal muscles without neurological signs or abnormal nerve conduction.

Of the 23 subjects originally classified as having 'probable/definite neuropathy', twelve (52%) were confirmed as having evidence of peripheral neuropathy. Four of these twelve had neurological signs and symptoms/abnormal QST and two also had abnormal EMGs. Eight of the 23 subjects (35%) had abnormal nerve conduction and symptoms and/or abnormal QST, six of whom had abnormal EMGs. A further three subjects had abnormal EMG without neurological signs or abnormal nerve conduction.

Thirteen (18%) of the 72 subjects had sensory abnormalities defined as abnormal sural nerve conduction and one or more abnormal QST values, while only two subjects (3%) had abnormal motor nerve conduction. Forty-seven subjects (65%) had abnormal small nerve fibre function, assessed by hot or cold sensation threshold, while 15 (21%) had abnormal large fibre function, assessed by vibration threshold or sural nerve conduction.

Neuropsychological findings

Subjects classified in the clinic as being 'probable/definite' cases of neuropathy had poorer self-reported general mental health and experienced greater self-reported anxiety and depression than other subjects less likely to have neuropathy.

Allowing for age and IQ, there was evidence of slower processing times among 'probable/definite' cases of neuropathy. However, on some measures across a variety of such tests the results were not consistent and did not provide clear evidence of an overall slowing of processing time.

Table A5.7: Neurological/Neurophysiological findings. Number of subjects with deficits/abnormalities (percentages)

Subject group	Number in group	Neurological signs	Nerve conduction	Neurological signs or nerve conduction	EMG	SFEMG	Abnormalities			
							Sensory	Motor	Small fibre	Large fibre
No neuropathy	15	0(0)	1(7)	1(7)	3(21)	0(0)	0(0)	0(0)	0(0)	1(7)
Possible neuropathy	34	3(9)	7(21)	10(29)	10(30)	5(15)	6(18)	0(0)	26(76)	7(21)
Probable/definite neuropathy	23	4(17)	8(35)	12(52)	11(52)	1(4)	7(30)	2(9)	21(91)	7(30)
Total	72	7(10)	16(21)	23(32)	24(35)	6(8)	13(18)	2(3)	47(65)	15(21)

Footnote:

The subject group is according to Phase 3 classification (based on assessment of neurological symptoms and QST measurement in the clinic).

Neurological signs:	Clinical assessment of reflexes, sensation and muscle power
Nerve conduction deficits:	Motor and/or sensory conduction in lower limb
Neurological signs or nerve conduction deficits :	Combination of the above. Those included here have a neuropathy in the clinical sense and would be reported as such to a referring GP
The following are used to characterise the neuropathy:	
EMG:	Needle electromyography of a muscle in the foot (EDB). The number in the groups was 14, 33 and 21 for 'no neuropathy', 'possible neuropathy' and 'probable/definite neuropathy' respectively
SFEMG:	Single fibre EMG
Sensory abnormalities:	Abnormal sural (sensory) nerve conduction **and** one or more abnormal QST thresholds
Motor abnormalities:	Abnormal conduction in common peroneal (motor) nerve
Small fibre abnormalities:	Abnormal hot or cold sensation threshold
Large fibre abnormalities:	Abnormal vibration sensation threshold or abnormal sural nerve conduction

Also, allowing for age and for general IQ, there was no evidence of a difference in memory ability between probable cases of neuropathy and 'no neuropathy' controls.

The results did not show that the neuropsychological findings were related to cumulative exposure to OPs, but it was acknowledged by the authors that the study design would have limited power to examine such a relationship.

Critique

Phase 1

Phase 1 of the study explored those factors that influence uptake of OPs by individuals. The results of phase 1 provide strong evidence that in sheep dipping the main determinant of individual dose of OPs is the handling of concentrate. In comparison, handling of dipped sheep during the dipping process was much less important. It is not clear whether the precautions taken to prevent exposure during the study were representative of practice in general.

Organophosphates **Phase 2**

With regard to the relationship between OP exposure and health, the original intention had been to use a neuropathy score based on the field survey in phase 2 as the main outcome and then validate it against a score derived in a similar way from the clinical assessment made in phase 3. Unfortunately, the neuropathy scores calculated in phase 2 were an unsatisfactory index of neuropathy, since there was poor agreement with the findings in the phase 3 clinical assessment. In particular, the clinical reference data were judged to be inappropriate for comparison with the QST measurements made in the field. This resulted in large numbers of subjects in all of the occupational groups being classified as abnormal. The investigators proposed that differences in ambient temperature might have accounted for the anomaly through an effect on limb and core temperature. The Working Group considered that, while an effect on altered limb temperature is plausible, the suggestion regarding core temperature is less so, and the possibility of other differences in recording techniques compared with those used for the clinical reference values (collected 10 years earlier) could not be excluded.

Whatever the explanation, the investigators rightly chose not to use neuropathy scores derived from the field survey as a health outcome. Instead they analysed symptom scores and the findings from the three QST tests separately, making comparisons between subjects according to their exposure to OPs and other characteristics.

One part of the analysis entailed comparisons between the three occupational groups sampled. The most striking differences observed were in the reporting of symptoms, the prevalence of which was positively associated with age, and also markedly higher in English than in Scottish farmers. The latter discordance suggests that there were important determinants of symptoms which the study did not assess directly, possibly related to an individual's awareness of, and his or her threshold for reporting, medical complaints. After adjustment for age and country, the prevalence of symptoms in sheep farmers was higher than that in ceramics workers, but was little different from that in non-sheep farmers. Given that this association was found in relation to only one of the two control groups, and also the possibility of confounding by unassessed determinants of symptom reporting, it is not possible to attribute the differences between occupational groups to an effect of OPs with any confidence.

Of the three QST measurements, the thresholds for heat and vibration sensation showed inconsistent differences between the three occupational groups, and only for cold sensation were thresholds higher in the sheep farmers than in either set of controls. The differences were not large and, in the context of the multiple statistical testing performed, they could have occurred by chance.

Another part of the analysis involved an internal comparison of sheep farmers according to their estimated exposure to OPs. This was a stronger part of the study, especially where exposure was related to QST measurements, which are more objective than reports of symptoms. It is of note, therefore, that none of the QST measurements showed a positive relationship with estimated cumulative exposure to OPs; this was the index of exposure believed *a priori* to be the most relevant. A statistically significant association was found

between symptoms and cumulative exposure, but this depended on a small number (four) of highly exposed individuals.

In addition, the investigators found that symptoms and vibration thresholds were significantly related to the extent of handling concentrate. In particular, symptoms were 3.4-fold (95%CI, 1.63 to 7.23) more common in farmers who had handled concentrate at some time. However, the risk did not increase progressively with the duration of handling the concentrate, as might have been expected if the association represented a toxic effect. This finding merits further investigation in future research but at present should be regarded as generating a hypothesis.

Phase 3

The results of phase 3 highlight the unreliability of the neuropathy score based on measurements made in the field in phase 2. The sampling strategy for phase 3 did not allow direct validation of the four health outcomes that were eventually used in phase 2. In addition, it was not possible to relate the findings in phase 3 to OP exposures. There was an excess of neuropsychological abnormalities in subjects who showed evidence of neuropathy in phase 3, but the classification of neuropathy depended in part on the presence of relevant symptoms, and it is known that psychosocial variables strongly influence the reporting of many symptoms. Thus, the association could reflect, in part, a tendency for mildly anxious or depressed individuals to complain of neurological symptoms more readily, and does not necessarily signify two related toxic effects of OPs.

Overall conclusion

The findings of the IOM study are an important contribution to our knowledge. However, the study does have the limitations described, and the Working Group did not consider the findings to be definitive. The results must be considered with those of other studies that have looked at similar health outcomes. In common with all studies based on cross-sectional samples of working farmers, the IOM investigation was not designed to evaluate severe health effects that would prevent people from working.

Appendix 6
List of those who had meetings with members of the Working Group

22 May 1998
Dr DE Ray

8 October 1998 *
Mrs E Sigmund
Mr W Sigmund
Ms S Leather
Mr E Owen-Jones

13 October 1998
The Countess of Mar

14 December 1998*
Mr J Coyte
Mr D Hassall

14 December 1998*
Mrs J Wheatley

15 February 1999*
Mrs E Chapman
Ms A Craig

17 February 1999*
Dr G Jamal
Dr D Davies
Dr S Hodges
Dr V Howard
Dr P Julu
Professor A Watterson

16 March 1999*
Mr P Tyler MP
Mr E Llwyd MP
Mr C Gill MP
Dr I Gibson MP
The Countess of Mar

4 June 1999*
Mrs S Bray
Mr G Cleverton

29 June 1999*
Mr R Cooke
Mr P Dobson
Dr D McEwan

20 July 1999
Dr A Pilkington
Mr F Hurley
Dr D Buchanan
Mr S Hansen

* Denotes meetings with some members of the OP Working Group.

Appendix 7
List of those who made written submissions to the Working Group

Date	From	Contents
06/07/98	Dr J Britton Respiratory Medicine Division City Hospital Nottingham	Published paper – 'Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia' (The Lancet 1997).
07/07/98	Mr A R Lyons Department of Orthopaedic and Accident Surgery University Hospital Nottingham	Letter stating that there is ongoing joint research at Universities of Nottingham and Sheffield on this subject but no details provided. Letter states work won't be completed by 31st July 1998 (original deadline given for submissions)
07/07/98	The Countess of Mar House of Lords	Letter detailing personal experience of the adverse health effects of OPs. A number of published and unpublished papers; 'Psychoneuroimmunology:interactions between the nervous system and the immune system' – The Lancet 1995. 'Chlorpyrifos (dursban) associated birth defects: a proposed syndrome, report of four cases and discussion of the toxicology' – Int J Occup Med & Toxicol 1995. 'Organophosphate pesticides- neurological and respiratory toxicity' – Toxicol & Ind Health 1995. 'The T- and B- systems of immunity in pesticide intoxication sufferers'. 'Pathophysiological studies of neuromuscular function in subacute organophosphate poisoning induced by phosmet' – J Neurology , Neurosurgery and Psychiatry 1993. 'Chronic neurological effects of organophosphate pesticides' – BMJ 1996. Pesticide Action Network North America Updates Service citing a report by the North West Coalition for Alternatives to Pesticides (NCAP) NCAP report entitled 'Worst kept secrets: toxic inert ingredients in pesticides'. 'One-Man's suffering spurs doctors to probe pesticide-drug link ' -article from Wall Street Journal 1991.

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Date	From	Contents
16/07/98	The Pesticides Trust	List of references on chronic ill health effects of OPs from Pesticides Trust Research Database. Paper produced by the Trust on OPs and Sheep Dips. Copy of Pesticides News. Copy of Pesticides Trust Review 1997.
17/07/98	Novartis Animal Health UK Ltd	Letter citing two possible sources of information for the COT Working Group; The EHC document on diazinon (1998) and the ECETOC review entitled 'OP pesticides and their long-term effects on the nervous system' which is due to be published in August. Letter also comments on IEH report and Dr Jamal's work.
19/07/98	Richard Bruce Isle of Wight	Letter detailing personal experience of the adverse health effects of OPs .
20/07/98	OP Information Network Cornwall	Letter citing various Government documents on OPs.
21/07/98	Dr A Spurgeon Institute of Occupational Health University of Birmingham	4 published papers: 'Organophosphates:The Relationship Between Chronic and Acute Exposure Effects' – Neurotoxicology & Teratology 1996. 'Abnormalities on neurological examination among sheep farmers exposed to organophosphorus pesticides' – Occup Environ Med 1996. 'Modifiers of non-specific symptoms in occupational and environmental syndromes' – Occup Environ Med 1996. 'Neuropsychological effects of long-term exposure to organophosphates in sheep dip' – The Lancet 1995.
22/07/98	Help Organization – Organophosphate Poison Sufferers (HOOPS), Glasgow	Letter detailing personal experience of the adverse health effects of OPs.
22/07/98	US EPA	Copy of an EPA review of chlorpyriphos. The review contains an Appendix dealing with epidemiology studies of chronic neurobehavioural effects of organophosphates.
23/07/98	OP Information Network Cornwall	Copy of paper entitled 'Long-term effects of organophosphate pesticides' – Human & Experimental Toxicology.

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Date	From	Contents
24/07/98	Pesticides Exposure Group of Sufferers (PEGS) Cambridge	Statement to the COT from Mrs Enfys Chapman. Copy of statement prepared by Mrs Enfys Chapman for the Working Part meeting on 30 June 1997 at the Royal College of Physicians. Copy of statement to ?Pesticides Forum. 10 published papers; 'Anxiety Associated With Exposure to Organophosphate Compounds ' – Arch Gen Psychiatry 1976. 'Political, Economic and Philosophical Aspects of Pesticide Use for Human Welfare' – Reg Tox & Pharm 1984. 'Psychiatric sequelae of chronic exposure to organophosphorus insecticides' – The Lancet 1961. 'Angina pectoris caused by coronary microvascular spasm' – The Lancet 1998. 'Agricultural Chemical Use and Congenital Cleft Lip and/or Palate' – Arch Environ Health 1981. 'A case-control study of brain gliomas and occupational exposure to chemical carcinogens: the risk to farmers' – Am J Epidemiol 1998. 'Childhood leukemia and parents occupational and home exposure' – JNCI 1987. 'Women and the environment: a study of congenital limb anomalies' – Community Health Studies 1986. 'Congenital limb reduction defects in the agricultural setting' – AJPH 1988. 'The breathless farm worker' – BMJ 1984.
25/07/98	Mrs S H Bray Devon	Letter detailing personal experience of the adverse health effects of OPs.
?	Mrs B Sutcliffe Lancashire	Copy of Mrs Sutcliffe's submission to BSE Inquiry.
26/07/98	Mr J Fryer Bristol	Letter commenting on the use of OPs.
27/07/98	British Agrochemicals Association Ltd Peterborough	Letter commenting on the use of OPs.
28/07/98	Mr A R Bruce Isle of Wight	Further comments on the use of OPs.

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Date	From	Contents
29/07/98	Dr F M Williams Dept of Environmental & Occupational Medicine University of Newcastle	Details of the research carried out by the Neurotoxicology Group at University of Newcastle. 10 published papers & abstracts 'Interindividual variations in enzymes controlling organophosphate toxicity in man' – Human & Experimental Toxicology 1992. 'Electrophysiological and biochemical effects following single doses of organophosphates in the mouse' – Arch Toxicol 1994. 'Comparative studies of two organophosphorus compounds in the mouse' – Toxicology Letters 1995. 'Effects of multiple doses of organophosphates on evoked potentials in mouse diaphragm ' – Human & Experimental Toxicology 1997. 'Effects of multiple low doses of organophosphates on target enzymes in brain and diaphragm in the mouse' – Human & Experimental Toxicology 1997. 'Effect of pirimiphos-methyl on proteolytic enzyme activities in rat heart, kidney, brain and liver tissues in vivo' – Clinica Chimica Acta 1997. 'Acute effects of single doses of an organophosphorus compound, diazinon, on acetylcholinesterase activity and miniature endplate potentials in the mouse (abstract) – Human & Experimental Toxicology 1998. 'A comparison of the electrophysiological effects of two organophosphates, mipafox and ecothiopate, on mouse limb muscles' – Toxicology & Applied Pharmacology 1998. 'Cytochrome P450 isozymes involved in parathion metabolism by the liver (abstract) – Human & Experimental Toxicology 1997. 'Activation and detoxification of phosphorothioate pesticides by human liver microsomes' – BTS 1998.
03/08/98	Mr J Fryer Bristol	Further comments on the use of OPs.
06/08/98	Mr J Fryer Bristol	Further comments on the use of OPs.
22/08/98	Mr G Westcott Minehead	Letter detailing personal experience of the adverse health effects of OPs.
22/08/98	Mrs S H Bray Devon	Further comments on the use of OPs.

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Date	From	Contents
22/08/98	Mrs J Wheatley Maidenhead	Letter citing number of documents for consideration: HSE Guidance Note MS17. Notes on diagnosis of prescribed diseases C3 Poisoning by phosphorus. Report of 5th Agricultural Select Committee Investigating the efficiency and effectiveness of PSD and VMD. Extract from Henly Standard. Speech given by Mrs Wheatley at International Conference on the Agriculture, Fisheries and Agroindustry in the Mediteranean : March 1997. 'Mortality of farmers and farmers' wives in Egland and Wales 1979-80, 1982-90' Occup & Environ Med 1996. Chapter on delayed lesions caused by organophosphorus CW agents. 'Anticholinesterase used in the USSR: poisoning, treatment and preventative measures' . Information on Nuvan Top.
24/08/98	Mrs A Crocker Okehampton	Letter detailing personal experience of the adverse health effects of OPs.
25/08/98	Miss B Scammell Department of Orthopaedic and Accident Surgery University Hospital Nottingham	Letter.
01/09/98	Dow AgroSciences	Letter and copies of the following papers: 'Update of the morbidity experience of employees potentially exposed to chlorpyrifos' Occup Environ Med 1998. 'Chlorpyrifos Exposure and Human Health, Final Report of an Independent Panel of Scientists Convened by Dow Elanco in Cooperation with the US Environmental Protection Agency. Request for proposals to investigate potential neurobehavioral function following human exposure to the organophosphate insecticide, chlorpyrifos, DowElanco. Chlorpyrifos:Lack of Cognitive Effects in Adult Long-Evans Rats – pre-publication copy. Determination of the reference dose for chlorpyrifos: Proceeding of an Expert Panel – pre-publication copy.

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Date	From	Contents
08/09/98	Dr J W King Iver Heath	Report prepared by Dr King on the question of whether prolonged low level expsoure to OPs, or acute exposure to OPs of a lower dose than that causing frank intoxication, can cause chronic ill-health effects.
08/09/98	National Office of Animal Health Limited	Letter and copies of the following papers: OP sheep dips – NOAH briefing paper, Feb 1993. OP sheep dips -VPC advice, April 1993. List of papers available to enquirers from VPC, 1993. VPC paper on long term effects of OP compounds in humans. List of papers considered by VPC. OP sheep dips-summary prepared for VPC, October 1993. Government announcement on OP dips, December 1993. The use, range and availability of ectoparasite control products in the UK. D W Tarry & G L Coles. Diazinon Post Dipping Exposure in Humans, CVL study, October 1993. Occupational Hygiene Assessment of Exposure to Insecticides and the Effectiveness of Protective Clothing during Sheep Dipping Operations, IOM study, February 1994. OP sheep dips compulsory variations to product licences, NOAH appeal to VPC, November 1994. NOAH appeal to Medicines Commission, May 1995. Use of OP sheep dips.
07/10/98	Mrs S H Bray Devon	Letter with: Article from Daily Mail 7th Sept 98 Abstract from paper by P O Behan, J Nutr& Environ Med 1996 NFU article Health & safety data sheet on Sheep Dip Press articles on head lice treatments containing malathion Health & safety data sheet on Tiguvon 10 Press articles Letter from HSE to Mrs Bray
08/10/98	Mr J Fryer Bristol	Letter commenting on use of OPs
08/10/98	OP Information Network Cornwall	Presentation for the DoH – COT Sub-committee on OPs

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09/10/98	OP Information Network Cornwall	Extract from Medical Manual of Defence Agents, MOD Extract from Royal College of Anaesthetists Newsletter
09/10/98	Mr AR Bruce Isle of Wight	Letter on pesticide poisoning
12/10/98	OP Information Network Cornwall	Copy of NFU press release calling for centre of expertise for OP victims
13/10/98	National Office of Animal Health Ltd Enfield	Letter containing information of usage of OPs Copy of NOAH submission to BSE Inquiry concerning link between OPs and BSE.
13/10/98	OP Information Network Cornwall	Letter on trials of galanthamine hydrobromide trials in UK.
14/10/98	OP Information Network Cornwall	Letter and fax detailing two case histories.
14/10/98	Dr J A Munro Breakspear Hospital Hertfordshire	Copy of data submitted to Royal College of Physicians Working Party, July '97.
26/10/98	OP Information Network Cornwall	Extract from 'Notes on the Diagnosis of prescribed diseases' HMSO
19/11/98	The Countess of Mar	Letter and observations of the Countess of Mar on the Clinical Aspects of Long-term Low-dose Exposure
25/11/98	Mrs J Wheatley Maidenhead	Further papers to add to original submission of 22/08/98: Submission to the Environment Committee of the European Commission Initial statement to the BSE enquiry BSE enquiry. Excerpts from the transcript of proceedings Statement of Dr Steven Watley to the BSE enquiry NFU meeting papers for Berks Bucks and Oxon Executive Committee
27/11/98	OP Information Network Cornwall	Points addressed in MS17 – Medical aspects of work related exposures to organophosphates
8/12/98	Dr JW King Iver Heath	Supplementary to submission of 8/9/98 on the question of whether prolonged low-level exposure to OPs or acute exposure to OPs of a lower dose than that causing frank intoxication, can cause chronic ill-health effects

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Date	From	Contents
9/12/98	Elizabeth Sigmund OP Information Network Cornwall	Enclosures: Cancer in offspring of parents engaged in agricultural activities in Norway: incidence and risk factors in the farm environment (Int J Cancer Jan 1996) Birth defects among offspring of Norwegian farmers, 1967 – 1991 (Epidemiology Sep 1997) US Farm Children face risk of Serious Harm from Pesticides
11/12/98	Mr John Coyte Ivybridge	Statement and photographs
21/12/98	Phil Young Regional Drug and Therapeutics Centre Northern and Yorkshire NHS Executive	Enquires to NPIS on low dose exposure to Organophosphates
31/12/98	Dr Sarah Myhill Powys Wales	List of OP patients and their medical problems and article entitled 'Evidence of Adverse Effects of Pesticides' (Journal of Nutritional and Environmental Medicine – 1995).
6/1/99	Mr P Stocker Soil Association Producer Services	Report on the use of Sheep Dips 'No Room for Complacency' and 'The Organic Food and Farming Report'
6/1/99	Mrs J Wheatley Maidenhead	Letter regarding remedial action required: Circulation of MS17 The education of our medical community into modern toxicology including synergistic awareness Setting up of Regional Toxicology Centres

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28/1/99	Ian Panton St Davids Pembrokeshire	Copy of letter to the Rt Hon Frank Dobson, brief overview paper, The Health of our Nation – Organophosphate Poisoning and Enclosures: Toxic Chemicals in Agriculture Report to the Minister of Agriculture and Fisheries of the Working Party on Precautionary Measures against Toxic Chemicals used in Agriculture – 1951. Health and Safety Guidelines Note MS17. 'Health surveillance of Workers exposed to organo-phosphorus and carbanate pesticides. Department of Social Security 'Notes on the Diagnosis of Prescribed Diseases C3 – Poisoning by Phosphorus. Synopsis of Judgement from Hong Kong Mr Kristan Bowers Philips.
10/2/99	Ernie Patterson Northern Ireland Organophosphorus Sufferers Association	Report compiled by the Agriculture Committee of the Northern Ireland Forum, entitled 'Organophosphate Insecticides – their Use by the Farming Community'
17/3/99	Countess of Mar	Letter regarding concerns over research at the IOM. Also enclosed – an article from the Los Angeles Times 'Pesticides May Harm Brain'
17/3/99	Dr Helen Fullerton Farming and Livestock Concern UK	Enclosed paper 'Sensation: Clue to the Pathology of Chronic Organophosphate Poisoning. A Review of the Evidence with Case Reports'
7/4/99	Mrs SH Bray Barnstaple Devon	Enclosed report 'Organophosphates poison insects and mammals primarily by phosphorylation of the acetylcholinesterase enzyme (AChE) at nerve endings'
9/6/99	Elizabeth Sigmund OP Information Network Cornwall	Analysis of OPIN database at 9 June 1999 and a report by the National Rivers Authority on 'The disposal of Sheep Dip Waste – Effects on Water Quality.'
25/6/99	Richard Bruce Hill Place Cottage, Yarmouth Isle of Wight	Letter about the report by the Royal College of Physicians and Psychiatrists and OP victim case study
14/7/99	Richard Bruce Hill Place Cottage, Yarmouth Isle of Wight	Letter about the problems with the medical and regulatory authorities and personal medical history.
30/7/99	Elizabeth Sigmund OP Information Network	Report of an incident which involved the death of hundreds of ewes and lambs after being dipped.

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Date	From	Contents
11/8/99	Richard Bruce Hill Place Cottage, Yarmouth Isle of Wight	Letter about the discussions of the IOM report, misinformation, disregard for truth and safety.
19/08/99	Helen Fullerton Farming and Livestock Concern UK	A case of Pirimiphos Methyl Poisoning
20/08/99	Elizabeth Sigmund OP Information Network	Letter from Mrs E. Sigmund to the Guardian about articles on the death of a Mr Ronald Maddison at Porton Down.
28/07/99	Mike Matthewson Schering-Plough Animal Health	Schering–Plough commentary of the IOM Technical Memorandum Series "Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers"
24/08/99	Joanna Wheatley	Submission to the BSE inquiry.
31/08/99	Alison Craig The Pesticides Trust	Letter giving some more details about the – PEX Action on Pesticide Exposure (formerly PEGS): PEGS DATABASE – which Enfys Chapman built up between
03/09/99	Philip Lowndes, Chief Executive Officer Novartis Animal Health UK	Letter to Elizabeth Sigmund.
14/09/99	Elizabeth Sigmund OP Information Network	Submissions from Mrs Sigmund (dated from 27 March 1998 to 29 July 1999). They include letters from farmers detailing personal experience of the adverse effects of OPs.

Appendix 8
Medical and scientific literature considered by the Working Group

Organophosphates: Chemistry, Fate and Effects, San Diego: Academic Press, 1992.

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Medical and scientific literature considered by the Working Group

Organophosphates

Appendix 9
Membership of the Working Group on Organophosphates

Chairman

Professor H F Woods BSc BM BCh DPhil FFPM FRCP (Lond. & Edin.)

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Professor P G Blain BMedSci MB BS PhD FIBiol FFOM FRCP (Lond. & Edin.)
Mrs J Brander BSc Hons (Public Interest Representative)
Professor D N M Coggon MA PhD DM FRCP FFOM FMedSci
Professor S Darby BSc MSc PhD
Mrs R Edwards (Public Interest Representative)
Professor R A C Hughes MD FRCP
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Professor A G Renwick BSc PhD DSc
Professor M D Rugg BSc PhD FRSE
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Secretariat

Mrs D Davey
R J Fielder BSc PhD RCPathDipTox — Scientific Secretary
J L Lighthill BA Hons — Administrative Secretary
Ms S A O'Hagan BSc Hons

Observers

J B Greig MA DPhil
G H Jones MInstAM
E Smales BSc MB BS FRCR (until 5 July 1999)
Mrs J R Walden (from 5 July 1999)

Professor R A C Hughes, Professor A G Renwick and Dr J A Vale declared non-personal non-specific interests in this topic during the meetings of the Working Group.

Appendix 10
Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor H F Woods BSc BM BCh DPhil FFPM FRCP (Lond. & Edin.)

Members

Professor P J Aggett MB ChB FRCP MSc DCH FRCPCH
Professor N A Brown BSc PhD
P Carthew BSc MSc PhD FRCPath
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M Joffe MD MSc(Econ) FRCP FFPHM
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Secretariat

T Barlow BSc PhD
C C Boyle BSc MSc PhD DipTox
J B Greig MA DPhil — Scientific Secretary
J L Lighthill BA Hons — Administrative Secretary
Ms C A Mulholland BSc
J Shavila BSc MSc PhD

At the meeting of the Committee at which this report was discussed interests in the topic were identified as follows: Professor K J Chipman and Professor A G Renwick declared non-personal non-specific interests, Professor P J Aggett and Professor N A Brown declared personal non-specific interests, Dr I Kimber declared a personal specific interest and was excluded from the discussion and decision making.