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HISTORICAL RESEARCH REPORT

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1999

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 neurophysiological study

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WORLD HEALTH ORGANISATION
COLLABORATING CENTRE
FOR OCCUPATIONAL HEALTH

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INSTITUTE OF OCCUPATIONAL MEDICINE

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

by

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EPIDEMIOLOGICAL STUDY OF THE RELATIONSHIPS BETWEEN EXPOSURE TO ORGANOPHOSPHATE PESTICIDES AND INDICES OF CHRONIC PERIPHERAL NEUROPATHY AND NEUROPSYCHOLOGICAL ABNORMALITIES IN SHEEP FARMERS AND DIPPERS

OVERARCHING SUMMARY

BACKGROUND

In 1995 the Health and Safety Executive (HSE), the Department of Health (DoH) and the Ministry of Agriculture, Fisheries and Food (MAFF) jointly commissioned a major epidemiological study into the effects of long-term exposure to OP sheep dips. This study was carried out between November 1995 and April 1999 by the Institute of Occupational Medicine in Edinburgh (IOM) and the Institute of Neurological Sciences in Glasgow (INS).

The broad aim of the study as a whole was to investigate whether cumulative exposure to sheep dip OPs is related to clinically detectable measures of polyneuropathy. The aim of Phase 1 was to develop a model for uptake of organophosphates (OPs) based on simple task, procedural and behavioural aspects of sheep dipping, and to validate the model by comparisons with OP urinary metabolites during various dipping procedures. The OP uptake model from Phase 1 was applied to retrospective exposure data collected in the Phase 2 field study.

The specific objective of Phase 2 was by means of a cross-sectional field study of sheep farmers and dippers to study the relations between cumulative exposure to OPs, and clinically relevant indices of peripheral neuropathy.

The specific objectives of Phase 3 were to: classify in terms of clinical disease the subjects with abnormal indices of peripheral neuropathy identified in the Phase 2 field studies; describe any associations between neurological and neuropsychological abnormalities; and examine any evidence for a relationship between neuropsychological status and estimated cumulative OP exposure.

PHASE 1

Methods

The study involved one day surveys of twenty dipping sessions at farms mostly located in the Scottish Borders. Each survey involved observation and recording of the activities performed by individuals including: the frequency and extent of handling the concentrate dip; the extent and time of contact with dip wash (working strength dip); protective clothing worn; hand washing; smoking and eating habits, and any other significant incidents. Sheep dippers were also asked to provide urine samples before and after work. These were used to measure metabolites of diazinon to enable an estimate of uptake to be made.

Results

The study found that the most important source of exposure to OPs was contact with concentrate 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. Levels of urinary metabolites were found to increase with the frequency of handling of the concentrate containers. Larger flock sizes tended to result in more replenishment of the bath and hence more handling of the concentrate. Generally one person at each farm had responsibility for handling concentrate dip, usually the paddler, the individual responsible for submerging the sheep in the dip wash.

Increased splashing with dip wash was found to be positively associated with increment in urinary metabolites for a subset of individuals who had not been exposed to concentrate dip. Splashing with dip wash was related to proximity to the dipping bath. The study confirmed the results of the earlier work (Niven *et al*, 1993 and Niven *et al*, 1996) particularly in relation to concentrate being the most important source of exposure.

The model
The model proposed for the uptake of OPs during a full sheep dipping session was as follows:-

$$\text{Uptake} = a^{*}\text{CONC} + b^{*}\text{DIP}$$

The model requires inputs from the two important sources of exposure identified in the study, CONC representing concentrate and DIP representing dip wash. CONC is the expected number of times concentrate is handled. DIP is the expected time weighted splash score had an individual been 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 inter-individual variation and other unconfirmed sources of exposure could have a significant effect on uptake.

Key findings Phase 1

- The most important source of exposure to OPs was contact with concentrate dip which was almost always on the hands. Levels of urinary metabolites increased with increased handling of the concentrate containers.

- Increased splashing with dip wash was found to be positively associated with increment in urinary metabolites for a subset of individuals who had not been exposed to concentrate dip.

PHASE 2

Methods
For practical reasons it was decided to base the study on two areas of the UK where there is a relatively high density of sheep farming. The areas chosen in England were Hereford and Worcester, and the Borders, Lothian and Ayrshire in Scotland. Suitable farms were identified from a sampling frame constructed from databases of annual census data maintained by the Ministry of Agriculture, Fisheries and Food (MAFF) for farms in England and Wales and by the Scottish Office for farms in Scotland.

Of the 995 sheep farm owners sent letters of invitation, 61% initially agreed to participate in the study. The study group consisted of 612 farmers with sheep-dipping experience (SD farmers), 53 farmers with no sheep dipping experience (NSD farmers and farm workers) and 107 ceramics workers.

Retrospective exposure information was obtained for the period of common usage of OPs (1970 onwards), using a questionnaire developed during the first phase of the study. The exposure history questionnaire was developed on the basis of relatively stable and easily identifiable features of the sheep dipping roles, (i.e. shown to be related to uptake in Phase 1 of the study, and considered amenable to recall at survey in Phase 2.) The main features included were flock size; concentrate handling; and principal task/job.

Neurological assessments were conducted using a symptoms questionnaire in conjunction with a series of quantitative sensory tests (QST) based on the Mayo Clinic Methodology (Dyck *et al*, 1980). For this study,

the design of the questionnaire was modified as it was to be administered to farm workers in the field by a trained technician. Two automated tests for thermal sensation (hot and cold) and another for vibration sensation were also included. All three tests had demonstrated high sensitivity and specificity in a laboratory setting with controlled ambient temperature. Identification of symptoms and signs likely to represent peripheral neuropathy was based on the questionnaire and test results according to predetermined criteria.

Results

Among SD farmers, most subjects had experienced fewer than 100 days dipped (median 54 days), although a small number of individuals had experienced over 1000 dipping days. Total dipping days was highly correlated with the model-based exposure index (r=0.92) together with the cumulative concentrate handling and splash score components. Age at survey was not correlated with any of the exposure indices.

Unexpectedly high numbers of the study group were found to have abnormal sensory thresholds based on hospital clinical reference values, possibly because of the cold ambient temperatures at the time of the field surveys. It was decided that the use of the clinical reference values to detect abnormality in thresholds measured in the field could not be justified, and that the symptoms score and the three sensory test thresholds would be analysed separately in relation to exposure in the field study. The symptom score in particular, proved reasonably reproducible between field and clinic.

The crude prevalence of reported symptoms overall was highest among SD farmers (19%), followed by NSD farmers (11%) and ceramics workers (5%). In all groups autonomic symptoms were more frequently reported than sensory or motor symptoms.

Age was also found to be positively related to all three sensory test thresholds. In addition, males had higher thresholds, on average, than females. Adjusting for age and sex there were inconsistent differences among the occupational groups between the two countries for both hot and vibration thresholds, whilst for SD farmers cold thresholds were, on average 1.35× higher than among ceramics workers, and 1.65×higher than among NSD farmers.

Adjusting for the important confounding variables, among the four neurological response variables, only for symptoms was there evidence of a positive relationship with cumulative exposure. Further analysis of exposure effects revealed that the average concentrate handling intensity, independent of duration of exposure, could explain the difference between SD farmers and ceramics workers in relation to both symptoms reporting and cold threshold. For symptoms, those had ever acted as principal concentrate handler reported more symptoms than those who had not (OR=3.4; 95% CI 1.6—7.2). There remained a much higher prevalence of symptoms among English subjects compared to Scottish subjects (OR=2.0).

There was also some effect of concentrate handling intensity on all three sensory test thresholds that was more marked for cold and vibration thresholds. This effect rose from zero intensity and peaked at around the mid-point of the intensity range (4 handling events per day).

Key findings Phase 2

- Results showed higher rates of symptoms between OP exposed sheep dippers as a group compared with non-exposed workers. The associations between symptom score and various indices of cumulative exposure to OPs, suggest that in at least some of the sheep farmers and farm workers reported symptoms are due to exposure to sheep dip chemicals. Sensory symptoms were more commonly reported than motor symptoms by sheep dippers in the field study.

- The critical exposure factor seems to be contact with concentrate in that markedly higher rates of reported symptoms (adjusted for other factors) were reported among those who had at some time

been principal concentrate handlers. These differences generally disappear when non-exposed groups are contrasted with dippers who had not principally handled concentrate.

- There was no evidence that cumulative exposure to OPs was associated with impairment of measured sensory thresholds. The results suggest a relationship between QST measurements and exposure to concentrate but these are difficult to interpret. The possibility of an associated sensory neurophysiological component to the suggested symptom effect should therefore not be discounted.

PHASE 3

Methods

A subset of subjects involved in the Phase 2 field study were invited to participate in the Phase 3 clinical studies at the Institute of Neurological Sciences (INS) in Glasgow. Recruitment was carried out by the IOM. Seventy nine subjects attended assessments at INS, and 76 were included in the study group, comprising 17, 36 and 23 subjects respectively from the 'no', 'possible' and 'probable/definite' categories in the field study. All 79 were sheep farmers. No ceramics workers were invited to attend the clinical study, and of the few non-exposed farmers invited, none in fact participated.

The symptoms questionnaire used in Phase 3 was the same as that used during Phase 2 epidemiological survey, but excluded details of occupation or details of relevant occupational exposure, and was administered by a neurologist. This was followed by a clinical assessment based on the Mayo Clinic criteria. The same range of sensory tests (QST) were performed in Phase 3 as in the Phase 2 studies. Additional tests included nerve conduction and electromyography. A battery of neuropsychological tests was performed to assess the following functions; General Intelligence; Psychomotor Function; Attention ; Memory; Mood and Affect.

Results

Twenty three (32%) out the 72 subjects had confirmation of their neuropathy by neurological signs or nerve conduction abnormality. Ten (29%) of the 34 individuals classified as having 'possible neuropathy' had evidence of neuropathy. Three (9%) of these had neurological signs and symptoms/abnormal QST. One of the three also had abnormal EMG. The remaining seven (21%) showed symptoms/abnormal QST suggestive of neuropathy together with abnormal nerve conduction. A further six had abnormal EMG in distal muscles without neurological signs or abnormal nerve conduction.

Of the 23 subjects classified as having 'probable/definite neuropathy', twelve (52%) showed evidence of peripheral neuropathy. Four (17% of 23) of the twelve had neurological signs and symptoms/abnormal QST and two also had abnormal EMG. Eight (35%) had abnormal nerve conduction and symptoms/abnormal QST. Six of the eight had abnormal EMG. A further three had abnormal EMG without neurological signs or abnormal nerve conduction.

One (7%) of the 15 subjects from the 'no neuropathy' group had abnormal nerve conduction but no clinical (signs and symptoms) or QST evidence of neuropathy. Three subjects from this group had abnormal EMG

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

Autonomic nervous system (ANS) symptoms were reported more commonly than peripheral nervous system (PNS) symptoms in the phase 2 study. This is also the case in the phase 3 study for the 'no neuropathy' and

'possible neuropathy' groups. Sensory symptoms were more commonly reported than motor symptoms.

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 be diagnosed as having neuropathy.

Key findings Phase 3

- The neuropathy described in Phase 3 is predominantly of a sensory type both clinically and neurophysiologically and is characteristic of distal, chronic neuropathy with no acute features. Small fibre populations are affected more than large fibre populations. The results of the additional tests (clinical examination and nerve conduction) therefore corroborate the other aspects of the Mayo Clinic methods in detecting a possible toxic neuropathy in the clinical studies.

- Increasing likelihood of neuropathy, as based on symptoms and sensory tests in the clinic was associated with anxiety and depression as measured in the neuropsychological tests. The results did not show that the neuropsychological findings were related to cumulative exposure to OPs, but it was acknowledged that the study design would have limited power to examine such a relationship.

BIOLOGICAL PLAUSIBILITY

The results of Phase 2 showed that sensory symptoms were reported more commonly than muscle weakness. There was also a higher prevalence of abnormal sensory tests than expected, although the difficulties discussed above, of possible temperature-related artefacts and inappropriateness of clinical reference population, limit the reliability of this finding. Autonomic symptoms were also reported commonly by farmers compared with other groups, with earlier work suggesting an association between OPs and autonomic symptoms reporting.

In Phase 3, fifteen subjects had sensory abnormalities defined as abnormal sural conduction and one or more abnormal QST values. In contrast, only two subjects had abnormal motor nerve conduction and both were in the definite neuropathy group. The reproducibility for symptoms between Phase 2 and 3 suggested a similar pattern of reported symptoms.

Clinical experience suggests that a toxic neuropathy (i.e. that which might occur in association with substances such as OPs) is likely to affect the distal part of the lower limb first. Due to the relative susceptibility of sensory nerve fibres compared with motor nerve fibres, it is likely that sensory findings would predominate in chronic neuropathy of this type. Therefore for most of the subjects with evidence of neuropathy the findings support a toxic aetiology.

In relation to concentrate handling it is possible that exposures to concentrated forms of OPs above a certain threshold, on a repeated basis even over a relatively short timescale could be associated with long term health effects. The mechanism here could be similar to that seen with more acute poisoning, but with repeated sub-acute exposures producing an increasing proportion of non-reactivatable cholinesterase.

The acute cholinergic effects of OPs are caused by their ability to inhibit cholinesterases including acetycholinesterase, and various other esterases, resulting in widespread changes in function in the peripheral, central and autonomic nervous systems. Most of the symptoms and signs of acute poisoning are potentially reversible, and in general enzyme reactivation is quite rapid, although slowed considerably if enzymes are complexed to larger OP moieties.

Some types of OPs may continue to be released from body compartments potentially prolonging their action for months despite reactivation of the enzyme. It is then possible for a significant proportion of cholinesterase to be non-reactivatable, but this effect is not well established with OPs commonly used in the UK. Furthermore, OP compounds inhibit many other proteins and enzymes. The potential health effects of this inhibition are still mostly undetermined. However, these changes may play an important role in the pathogenesis of the chronic long term effects.

The results of the Phase 3 neuropsychological assessments suggest a subset of individuals with evidence of chronic neuropathy who also show evidence of anxiety and depression. OPs have also been linked with depression. It is considered that increased levels of acetylcholine in the central nervous system are associated with symptoms of depression. OPs have also been linked with depression. It is considered that increased levels of acetylcholine in the central nervous system are associated with symptoms indicative of anxiety or depression (Levin *et al*, 1976), and the greater prevalence of psychiatric morbidity mirrors the findings of an earlier study of sheep farmers (Stephens *et al*, 1995). Apart from acetylcholine, other neurotransmitters such as serotonin are probably involved.

OVERALL CONCLUSION

Considering the study as a whole the findings suggest an association between exposure to OPs, predominantly the concentrate, and evidence of chronic peripheral neuropathy. It is conjectured that exposures to concentrated forms of OPs above a certain threshold, without producing overt cholinergic poisoning, on a repeated basis could be associated with long term health effects. This is supported by Phase 1 findings, which suggested most uptake of OPs occurred in association with concentrate handling events., although a relationship between uptake and splashing with dilute dip wash was also found.

POSSIBLE RESEARCH IMPLICATIONS OF THESE FINDINGS

There is limited published evidence in the UK at present in relation to the effects of concentrate handling. Further clinical investigations or animal experiments may be necessary to explore the suggested hypothesis of subacute repeated exposures, possibly over a shorter timescale. However, as the mechanism by which OPs may produce health effects is still unclear, further exploration of other relevant hypotheses may also be justified.

Autonomic nervous system (ANS) symptoms were reported more commonly than peripheral nervous system symptoms. It is very difficult to assess function on the basis of questionnaire alone. However, earlier work suggests that the ANS may be selectively affected by toxic exposures such as OPs, and further detailed investigations of the autonomic nervous system may be warranted in those showing probable health effects in this study.

The results of this study suggested that increasing likelihood of neuropathy, as based on symptoms and sensory tests in the clinic was associated with anxiety and depression as measured in the neuropsychological tests. It was acknowledged that the study design had limited power to examine an exposure-response relationship. However, larger scale neuropsychological investigations may be warranted in view of the findings in those categorised as 'probable' neuropathy.

The possible regional differences in symptoms reporting between England and Scotland remain unexplained. It has been noted that public perception of a possible OP problem was stronger in England than in Scotland, and this too may have contributed to a difference in prevalence. However, comparison of symptoms reporting in Phase 2 and Phase 3 showed better reproducibility among sheep dippers in England than in

Scotland. It is reasonable to consider if there might be a substantial reason for the differences, although this does not appear to be associated with differences in product usage.

This study has also shown that the neurological symptoms questionnaire can be used reliably in a field setting by a trained technician. Whilst it was possible to perform sensory tests in the field, it would appear that these tests require more controlled conditions in order to produce reproducible results compared with clinical studies. Alternatively it would be useful to establish reference data for these tests based on field measures which adjust for variation in ambient temperature. However these problems highlight the importance of designing in an evaluation of the reliability of field measurements.

CONTENTS

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

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

SUMMARY

a. Summary of aims

In 1994, the then Minister of Agriculture, Fisheries and Food announced that the Government had accepted advice from the Medical and Scientific Panel of the Veterinary Products Committee that there should be further research into the effects on health of organophosphate (OP) sheep dips. Subsequently, the Health and Safety Executive (HSE), the Department of Health (DoH) and the Ministry of Agriculture, Fisheries and Food (MAFF) jointly commissioned a major epidemiological study into the effects of long-term exposure to OP sheep dips. This study was carried out between November 1995 and April 1999 by the Institute of Occupational Medicine in Edinburgh and the Institute of Neurological Sciences in Glasgow. The conduct and results of the research are being reported in three companion volumes, of which this report is the third.

The broad aim of the study as a whole was to investigate whether cumulative exposure to sheep dip OPs is related to clinically detectable measures of polyneuropathy. The specific objectives of Phase 3 were to: classify in terms of clinical disease the subjects with abnormal indices of peripheral neuropathy identified in the Phase 2 field studies; describe any associations between neurological and neuropsychological abnormalities; and examine any evidence for a relationship between neuropsychological status and estimated cumulative OP exposure.

This was achieved by a nested case-control clinical study of selected subjects with neurological abnormalities identified in the field studies, and of control subjects. This investigation aimed to provide additional information about the nature of any neuropathies found, and included a neuropsychological component which had not been practicable in the field investigation.

A recent report by the Royal Colleges of Physicians and Psychiatrists (1998) suggests that reports of chronic low dose effects of OPs are limited by small numbers of cases, selection bias and inadequate controls. It also considered that some cases may be the result of undocumented episodes of acute exposures. A review by ECETOC (1998) concluded that animal experiments confirm acute and protracted effects on cognitive function, but have not demonstrated effects of prolonged low level

exposure. Both these reports suggest that the issue of OP exposure and health remains important and controversial.

b. Methods

A subset of subjects involved in the Phase 2 field study were invited to participate in the clinical studies at the Institute of Neurological Sciences (INS) in Glasgow. Recruitment was carried out by the IOM. To ensure a wide representation of disease status in the clinical study, the objective was to select individuals in equal numbers from the 'no', 'possible' and 'probable/definite' groups as derived from the field survey data, using the original neuropathy score. Seventy nine subjects attended assessments at INS, and 76 were included in the study group, comprising 17, 36 and 23 subjects respectively from the 'no', 'possible' and 'probable/definite' categories in the field study. All 79 were sheep farmers. No ceramics workers were invited to attend the clinical study, and of the few non-exposed farmers invited, none in fact participated.

The symptoms questionnaire used in Phase 3 was the same as that used during Phase 2 epidemiological survey, but excluded details of occupation or details of relevant occupational exposure, and was administered by a neurologist. This was followed by a clinical assessment based on the Mayo Clinic criteria. The same range of sensory tests (QST) were performed in Phase 3 as in the Phase 2 studies. Additional tests included nerve conduction and electromyography. During the clinical assessments none of the INS personnel were aware of the subjects= occupational exposure or the neuropathy classification derived from the results of the Phase 2 field study.

A battery of neuropsychological tests was performed to assess the following functions; General Intelligence; Psychomotor Function; Attention ; Memory; Mood and Affect. General Intelligence was used as a control variable and measured using the National Adult Reading Test. A verbal IQ estimate was derived based on the number of words read correctly from a standard list.

The CANTAB battery was developed for the assessment of cognitive defects in humans with degenerative brain disease. CANTAB has been used in early identification of progressive neurological disorders, and in toxicological assessments for acquired disorders such as alcoholism. Standardised data are therefore available on normal volunteers for all the tests (Robbins *et al.* 1994).

A questionnaire on recent exposure to OPs was administered to all Phase 3 participants by an independent nurse. The format used was similar to that of the Phase 2 exposure-history questionnaire. Five individuals had handled concentrate in the two weeks prior to the commencement of Phase 3.

c. Main findings

Reproducibility of symptoms and QST

The study was designed from the outset to include a detailed clinical examination of selected subjects who had earlier participated in the field surveys. On comparing the field (Phase 2) and clinic (Phase 3) classifications using the neuropathy scoring system, although agreement was better than chance, it was not considered reproducible enough as a basis for exposure-response modelling of disease status in the field. The symptom score component proved reproducible. The sensory thresholds were less reproducible, although this was felt to be due to comparison of thresholds measured in the field with an inappropriate reference population.

There were major inconsistencies between field study QST measurements and the clinical reference values. Measurements had been taken during training by all technicians. Some of these had been in the clinic and others outwith the clinic. None had indicated any likelihood of the problems found. However, none had been taken under the kinds of temperature extremes which occurred later during the actual field survey. There are strong grounds for believing that QST results may be sensitive to individuals' limb temperature and related core temperature.

Additional neurological tests

Three individuals had the following conditions recorded on their Phase 2 neuropathy questionnaires: rheumatoid arthritis, family history of high arches, and hypertension requiring medication which was associated with hypotension. It was decided to err on the side of caution and exclude them from remaining Phase 3 analyses to limit possible confounding. A further four subjects were found to have a profile of abnormalities of neurophysiological parameters not consistent with generalised neuropathy. Three had carpal tunnel syndrome and one subject had findings consistent with radiculopathy. These subjects, two each from of the 'no neuropathy' and 'possible neuropathy' groups, were excluded from further analysis. This left a total of 72 subjects for analysis.

The groupings described in the following paragraphs are all based on the Phase 3 neuropathy score for symptoms and QST. Twenty three (32%) out the 72 subjects had confirmation of their neuropathy by neurological signs or nerve conduction abnormality. One (7%) of the 15 subjects from the 'no neuropathy' group had abnormal nerve conduction but no clinical (signs and symptoms) or QST evidence of neuropathy. Three subjects from this group had abnormal EMG.

Ten (29%) of the 34 individuals classified as having 'possible neuropathy' had evidence of neuropathy. Three (9%) of these showed only clinical evidence including neurological signs and symptoms/abnormal QST. One of the three also had abnormal EMG. The remaining seven (21%) showed symptoms/abnormal QST suggestive of neuropathy together with evidence of abnormal nerve conduction. A further six had abnormal EMG in distal muscles without neurological signs or abnormal nerve conduction.

Of the 23 subjects classified as having 'probable/definite neuropathy', twelve (52%) showed evidence of peripheral neuropathy. Four (17% of 23) of the twelve had neurological signs and symptoms/abnormal QST and two of these also had abnormal EMG. Eight (35%) had of abnormal nerve conduction and symptoms/abnormal QST. Six of the eight had abnormal EMG. A further three had abnormal EMG without neurological signs or abnormal nerve conduction.

Thirteen (18%) of the 72 subjects had sensory abnormalities defined as abnormal sural conduction and one or more abnormal QST values while only two subjects (3% of 72) had abnormal motor nerve conduction and both were in the definite neuropathy group. Forty seven subjects (65% of 72) had abnormal small nerve fibre function, assessed by hot or cold sensation threshold, while only 15 (21% of 72) had abnormal large fibre function, assessed by vibration threshold or sural nerve function. Thus, small fibre dysfunction was three times more common than large fibre dysfunction. In defining the relative involvement of small versus large and sensory versus motor fibre populations, one must remember that methods used to assess them may not have identical sensitivity.

Autonomic nervous system (ANS) symptoms were reported more commonly than peripheral nervous system (PNS) symptoms in the phase 2 study. This is also the case in the phase 3 study for the 'no

neuropathy' and 'possible neuropathy' groups. Sensory symptoms were more commonly reported than motor symptoms.

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 be diagnosed as having neuropathy.

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

Also, allowing for age and for general IQ, there was no evidence of a difference in memory capability 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 that the study design would have limited power to examine such a relationship.

d. Key findings

- The neuropathy described in Phase 3 is predominantly of a sensory type both clinically and neurophysiologically and is characteristic of distal, chronic neuropathy with no acute features. Small fibre populations are affected more than large fibre populations. The results of the additional tests (clinical examination and nerve conduction) therefore corroborate the other aspects of the Mayo methods in detecting a possible toxic neuropathy in the clinical studies.

- Increasing severity of neuropathy, as based on symptoms and sensory tests in the clinic was associated with anxiety and depression as measured in the neuropsychological tests. The results did not show that the neuropsychological findings were related to cumulative exposure to OPs, but it was acknowledged that the study design would have limited power to examine such a relationship.

The implications of these findings are considered in more detail in a summary of all three Phases which can be found in this Phase 3 report.

1. INTRODUCTION

1.1 BACKGROUND

This report describes the third phase of an exposure-response epidemiological study investigating the effects of organophosphate pesticides (OPs) in sheep farmers and dippers. The third phase includes a nested study of the neurological, neurophysiological and neuropsychological status of cases with abnormal neurological indices, and controls with normal indices. The aim of the work was to facilitate the clinical interpretation of the associations found in Phase 2, refine any exposure-response relations found, and compare neuropsychological status with neurological status and OP exposure.

The first phase of the study (Sewell *et al*, 1999) included the development of an uptake model relating OP uptake to simple descriptions of task components observed during dipping practice. This was used to develop surrogate measures of exposure, from which retrospective exposure data were obtained during the second phase of the project (epidemiological field studies). Information was also obtained during the second phase on neurological symptoms and vibration and thermal thresholds were also assessed, to determine whether exposure-response relationships could be identified. Exposure was quantified on the basis of the OP uptake model. Subjects were classified according to predefined neurological criteria, and a subgroup has been selected for more detailed neurological and neuropsychological assessment in the third phase of the study.

Briefly, the work of Phase 3 included repeating the neurological assessments performed in the Phase 2 field studies. However, all assessments were performed by clinical staff in a hospital environment. A range of additional clinical and neurophysiological tests were used to refine the diagnostic category assigned as a result of Phase 2 investigations. Information was also obtained on neuropsychological status which, due to the test requirements, was not possible to establish during the Phase 2 field studies.

1.2 SUMMARY OF CURRENT KNOWLEDGE OF OP HEALTH EFFECTS

1.2.1 Acute cholinergic effects

The acute cholinergic effects of organophosphates are well documented and are mainly mediated through the suppression of the enzyme acetylcholinesterase which results in the accumulation of acetylcholine at the receptor site. There is also evidence from *in vitro* studies of an additional direct action of some organophosphorus pesticides and carbamates on cholinergic receptors. However, the relevance of this finding for clinical cases is not yet clear.

There is also evidence that tolerance can develop to the acute cholinergic effects of cholinesterase inhibitors (Richardson, 1995). In mammals resistance to the cholinergic effect of organophosphates is directly related to speed of metabolic inactivation and excretion, and is therefore influenced by individual metabolic capacity. This susceptibility is likely to apply to both acute and chronic exposure and relates to both inherent metabolic capacity and interactions with other xenobiotics, which share the same metabolic pathways. Organophosphorus esters are broken down by spontaneous hydrolysis and by esteratic, dealkylating and oxidative metabolism. It is difficult to determine which process is most important for a specific target tissue. Individual susceptibility is also affected by variation in liver detoxification capacity.

Less than 20 incidents of acute intoxication with OPs were reported per year between 1984 and 1987 (Weir *et al*, 1992) , from an estimated 300,000 people potentially exposed to sheep dip pesticides. However, the real incidence of mild-moderate acute OP poisoning is not clear since it is likely that most of these are missed or not reported (e.g. Dippers flu).

1.2.2 Organophosphate induced delayed neuropathy

In addition to the acute neurological effects, certain organophosphates are well recognized to be associated with a delayed neuropathy known as organophosphate induced delayed neuropathy (OPIDN). Clinical signs develop 2-3 weeks after exposure to the compound and consist of a distal symmetrical motor-sensory mixed peripheral neuropathy mainly affecting the lower limbs, and a central axonal neuropathy mainly affecting the spinal cord and brainstem structures initially producing weakness evolving to spasticity and ataxia. The delayed neuropathy is not thought to be related to inhibition of acetylcholine but associated with phosphorylation of neuropathy target esterase (NTE) followed by ageing in the enzyme complex. Ageing involves covalent cleavage of a bond within the enzyme-substrate adduct, and the formation of a negative charge which stabilises it. Once this has occurred the enzyme is irreversibly inactivated. The rate of this ageing reaction is also enzyme, and agent, specific. After ageing has occurred reactivity is only regained by synthesis of further enzyme. NTE is only sensitive to a small number of specifically "neuropathic" organophosphorus agents.

Distal axonal degeneration is the main and earliest feature of OPIDN and involves both peripheral and central motor and sensory fibres in relation to axonal length and diameter affecting motor more than sensory fibres. All the agents clearly linked with OPIDN in man have also produced neuropathy in hens. NTE when phosphorylated can be used as a marker for the development of a delayed neuropathy if this is followed by ageing of the phosphorylated enzyme complex. Determination of NTE activity in lymphocytes and ratio measures of NTE:AChE can be used in conducting safety assessments for OPIDN potential. Experimental studies in hens (Lotti and Johnson,1973) have shown that chronic multiple dose exposure to neuropathic organophosphates yields an NTE inhibition threshold for neuropathy which is only 10% lower than that following a single acute exposure.

It is generally considered unlikely that classical OPIDN could be produced by chronic low level exposure to OPs.

1.2.3 Intermediate syndrome

A paralytic intermediate syndrome has also been described with an onset of 24-96 hours without or after cholinergic effects lasting for about three weeks. The presentation is that of a proximal neuropathy with involvement of the cranial nerves and possibly the brainstem. Ventilatory failure may occur due to paralysis of the respiratory muscles. The precise pathophysiology is not understood, although the clinical course suggests the possibility of a demyelinating proximal neuropathy.

1.2.4 Possible long term effects of acute OP poisoning

Recent studies have also suggested that organophosphates can produce long term effects which may be secondary to one or more acute toxic episodes or as a result of chronic long term exposure to low doses of OPs insufficient to produce acute symptoms.

There is general agreement that long term effects can result following one or more acute episodes and epidemiological studies suggest a variety of long term consequences of exposure (Savage *et al* 1988, Rosenstock *et al* 1991, McConnel *et al* 1994 and Steenland *et al* 1994).

Savage *et al* (1988) reported 303 subjects accidentally poisoned, 141 met predefined criteria (alcohol use, age, lack recent exposure, neurological disease), 100 were eventually assessed. The control group was drawn from the same geographical area and matched for age, sex, years, education, SE group, occupation and race. Out of 39 psychological tests, 22 showed statistically significant differences in tests of memory and abstraction but no differences on EEG or neurological examination. Cases had poorer reading ability than controls, therefore educational differences may have contributed to the differences.

Rosenstock *et al* (1991) conducted a retrospective neuropsychological study of severe accidental poisoning. Of 89 identified cases, 38 were located and 36 agreed to participate. Controls were 36 age-matched friends or brothers of the subjects. Measurements were made 10-34 months after the poisoning episode and against a background of normal occupational pesticide exposure in both cases and controls. Cases showed a non-significant increase in reaction time and anxiety and non-significant decreases in verbal learning and finger tapping. There were also differences in vibrotactile threshold which could not be explained by variation in skin thickness. The threshold differences were greatest in cases involving methamidaphos, an agent known to cause OPIDN in man after severe intoxication.

McConnell *et al* (1994) studied the long term effects of acute OP poisoning on peripheral nerve function (assessed by vibro-tactile sensation measurement) in 36 subjects compared with an age and sex matched control community reference group. They reported abnormal peripheral nerve function in the poisoned group between 10-34 months after their acute poisoning episode. They concluded that 'reported cases of OP induced delayed neuropathy may represent only the worst disease in a spectrum of impairment, a sequelae of exposure that may be much more common than previously thought'.

Steenland *et al* (1994) studied a group of workers with either unambiguous signs of poisoning or a reduction in cholinesterase inhibition of at least 20%. In all, 128 cases were studied and 90 controls matched on race, lifestyle factors and solvent use. Controls were 4.3 years older than cases on average. There was a significant decrease in vibration sensitivity ($p<0.05$) for those with cholinesterase inhibition, with hospitalised cases showing the greatest change. After correcting for confounders, sustained visual attention was found to be reduced in all cases, with symbol-digit substitution being reduced in the hospitalised group.

1.2.5 Possible long term effects of low level exposure

Low level exposure can be defined as exposure at a level low enough not to produce any clinically detectable cholinergic features. The chronic effects on the peripheral and central nervous system are less extensively documented. It is difficult to relate effects to specific agents where occupationally exposed workers may be potentially exposed to a variety of pesticides and other agents. The phosphorylation of neuronal protein sites may contribute to the underlying disorder but genetic differences in detoxification of enzymes and non-specific binding is also known to account for some of the interindividual variation in susceptibility to anticholinesterases. Other enzyme systems or proteins may contribute to this effect.

Many studies have been published on the effects of long-term low level exposure and though the majority showed consistent correlation between exposure and effect, the results have not been as consistent as those following acute poisoning episodes. For example, Stephens *et al* (1995) studied 158 sheep dippers and 155 quarry workers chosen as a control group. None of the farmers had experienced acute poisoning and had on average been involved in sheep dipping for 15 years. The follow-up study involved seven neuropsychological tests and two health questionnaires. The authors found significant differences in sustained attention and speed of information processing in the exposed group but no effects on memory or learning. Basic neurological examinations were later performed on 10 symptomatic farmers, 10 asymptomatic farmers and 10 controls. These showed some signs of sensory disturbance but little evidence of gross motor dysfunction in the farmers.

Fiedler *et al* (1997) reports on a neuropsychological study of 57 fruit tree sprayers and 42 non-exposed farmers or shopkeepers. The sprayers had used OPs for a mean of 27 years. Estimated levels of pesticide exposure were low to moderate with no reported evidence of poisoning. Tests were conducted in the non-spraying season to avoid current exposure. The controls had on average been educated for longer, and had better reading ability. Although the study found reduced reaction time in high exposed individuals this was largely attributable to age, and there was no significant difference in other neurobehavioural tests.

Ames *et al* (1995) studied 45 agricultural pesticide applicators who used a variety of organophosphates, and who each had at least one documented episode of cholinesterase inhibition but were asymptomatic. The control group of 90 males were from the same geographical area and were 8.7 years younger on average than cases but otherwise well matched. They found no difference between groups in nerve conduction, vibration sensation, motor function or mood. Serial digit test scores were better in the exposed group. However, the authors found that these measures were changed by a higher level of exposure sufficient to cause acute poisoning.

Stokes *et al* (1995) measured vibration sensation in 90 orchard sprayers and 68 matched controls. The sprayers had used at least five OPs over a mean of 20 years. Vibration thresholds were measured out of season and showed a 75% increase in the finger thresholds, which was statistically significant ($p<0.01$), but not the foot. Of peripheral signs used in the studies performed to date, EMG decrement appears to be a sensitive measure of exposure which is relatively specific to anticholinesterase effects. The neuropsychological studies suggest that reaction time measures are more consistently impaired than performance.

Studies of neurobehavioural manifestations of OP intoxication are subject to methodological difficulties due to lack of control groups, small study populations, difficulty in ascertaining the exact nature of the exposure.

A limited number of studies have considered neurological health effects of long term low dose exposures to OPs have often only reported findings in small groups of subjects and have relied on basic assessments of neurological function. The nature of the exposure often varies considerably between different studies, as does the presence of significant co-exposures, and the availability of adequate personal protection. This makes it difficult to overview the findings of most published studies in a consistent manner. There is also the potential difficulty of interpreting non-specific symptoms in relation to mixed exposures.

1.3 OVERVIEW OF FIELD STUDY DATA (PHASE 2)

The Phase 2 study consisted of a cross-sectional comparison of exposure to sheep dip OPs and chronic peripheral neuropathy throughout the UK. For practical reasons it was decided to base the study on two areas of the UK where there is a relatively high density of sheep farming. The areas chosen in England were Hereford and Worcester, and the Borders, Lothians and Ayrshire in Scotland.

The final study group consisted of a total of 772 subjects. Of these, 107 were ceramics workers with no experience of sheep dipping, 53 were farmers or farm workers with no experience of sheep dipping, and 612 were farmers or farm workers with at least three days experience of sheep dipping.

Retrospective exposure assessments were obtained for the period of common usage of OPs (1970 onwards), using a questionnaire developed during the first phase of the study. Phase 1 of the study involved careful observations of task and working practice in relation to uptake of OPs as assessed by urinary OP metabolites.
Neurological assessments were conducted using a symptoms questionnaire in conjunction with a series of quantitative sensory tests (QST) based on the Mayo Clinic Methodology (Dyck *et al*, 1980).

The results of the Phase 2 field studies (Pilkington *et al*, 1999) have suggested that there was a significant difference in symptoms reporting between ceramics workers and sheep dipping farmers, which is larger than expected on the basis of age differences and only explained in part by cumulative exposure. There were similar smaller differences in cold threshold between the groups after adjustment for cumulative exposure. Sheep dippers also reported more symptoms than non-sheep dippers, and had higher cold thresholds, although these findings were not statistically significant.

There were also regional differences in symptom reporting between sheep farmers in Scotland and England with English farmers reporting more symptoms. Sheep dippers who handled concentrate had three times the reported prevalence of symptoms than non-concentrate handlers.

The associations between symptom score and various indices of long-term exposure to OPs, together with the patterns of symptoms reported, are fairly strongly suggestive that at least some of the symptoms in sheep farmers and farm workers are due to exposure to sheep dip chemicals. In the context of the study, this means principally exposure to organophosphate products. Indeed, the critical factor seems to be exposure to concentrate, though a relationship with days dipped was strongly suggested.

1.4 AIMS

This third phase of this study had the following aims:

(a) To classify in terms of clinical disease the subjects with abnormal indices of peripheral neuropathy identified in the Phase 2 field studies

(b) To explore what neuropsychological profiles, if any, may be associated with neurophysiological damage in the subjects studied;

(c) To explore what neuropsychological characteristics or profile, if any, may be associated with cumulative exposure to OPs.

There is a difference in the level of ambition associated with these three aims. The main purpose of Aim (a) was to classify in terms of clinical disease a range of subjects from Phase 2, selected from groups of 'no', 'possible', and 'probable/definite' neuropathy based on applying pre-determined criteria to results from the symptoms questionnaire and sensory tests carried out under field survey conditions in Phase 2. This was to allow a better interpretation of the nature of any neuropathies found. Results are in Chapter 6.

The detailed neurophysiological tests and examinations of Phase 3 made it possible however to establish or confirm the strengths and weaknesses of the Phase 2 neuropathy classification in two ways. The first was to confirm *reproducibility*, by comparing the Phase 2 classification (and the underlying measurements) with a classification based on similar measurements taken in the clinic under more controlled conditions and by specialist staff. Results are in Chapter 5. The second was to confirm its *validity*, by examining whether results from additional neurophysiological tests which were not practicable under field conditions were consistent with classifying subjects based on symptoms and sensory tests only (i.e. the types of measurements taken in Phase 2), though using the better Phase 3 data for symptoms and sensory tests.

These aspects might be considered as confirmatory in that there is already a strong body of evidence about neurophysiological measurement and classification of neuropathy, for example the Mayo Clinic methodology which underpins the present study. This is in contrast with neuropsychological tests, where there is no existing body of evidence from people exposed to OP products to indicate *a priori* what patterns of results might be associated either with neuropathy in OP-exposed individuals (Aim b) or with OP exposures directly (Aim c), although it was possible to make some *a priori* conjectures based on general considerations of what kinds of damage might be expected. The purpose of Aims (b) and (C) was therefore acknowledged to be exploratory, ie. by gathering and examining data in a structured way, to generate ideas or hypotheses whose confirmation would need further and larger studies, and which meantime would need to be considered as provisional. The exploratory nature of the neuropsychological work was indicated clearly in the original research proposal. Results are in Chapter 7.

1.5 STUDY DESIGN

The study comprised clinical investigations of subjects identified from the epidemiological study as possibly or probably neurologically abnormal, and of a representative sample of those without abnormality. Subjects were examined clinically according to a standardised procedure, and classified in clinical and neurophysiological terms according to internationally validated predetermined criteria.

Whilst there are established neuropsychological profiles for some conditions such as Alzheimer's disease, there are currently no such profiles characterising the potential neuropsychological effects of long-term exposure to OPs. Therefore, the neuropsychological variables to be recorded in Phase 3 have been classified into groups which are informative of different facets of an individual's functioning. The framework for this forms part of the normal grouping of CANTAB tests and took place prior to the data collection for Phase 3 (see section 2.3.2). The purpose of this grouping was to facilitate a structured exploration of the data. Which is appropriate in view of the current state of knowledge in this area of neuropsychological investigation.

The study design required to meet the three objectives outlined above differs and the respective approaches are discussed below.

1.5.1 A case-control study (Phase 3a and 3b)

The analysis is that of a case-control (case-referent) study, nested within the cross-sectional study of Phase 2. The 'cases' are subjects with definite or probable neuropathy in Phase 2; and the controls are those almost certainly without neuropathy in Phase 2. In addition, there is a third, intermediate, possible neuropathy group. The inclusion of this third group does not distort the main case-control design and the thrust of the analysis involves comparing and contrasting groups of subjects identified by (neurophysiological) disease status.

1.5..2 A small cross-sectional study (Phase 3c)

The analysis of this component of the study is not within a traditional case -control framework; ie. it is not a comparison between groups of cases and controls (and an intermediate group). Rather, it fits a conventional cross-sectional framework. In this case the outcome variables are neuropsychological measures and the principal explanatory variables are indices of past exposure to OPs. The thrust of the analysis is to examine relationships, if any, between explanatory and outcome variables, taking appropriate account of other (confounding) factors.

The case-control design of Phase 3 is in effect a stratified sampling procedure for Phase 3, where the strata are the Phase 2 groupings of neuropathy from which cases and controls were selected. This stratification will have influenced the distribution both of the neuropsychological status and the OP exposures of Phase 3 subjects, to the extent that OP exposure and neuropsychology are related to the Phase 2 grouping of neuropathy. It may similarly affect the apparent relationship between OP exposure and neuropsychology, so that estimates of that relationship would be biased unless the stratification by design was taken into account. Suitable adjustments were made in the course of analysis (see Chapter 7), so that relationships are explored only *within* Phase 2 neuropathy groupings, where they are unaffected by the case-control design. This, together with the small number of subjects, means that this aspect of the study has very limited power to detect effects, unless these are very strong.

1.6 STUDY POPULATION

Seventy nine subjects participated in the clinical studies. The selection process is described in more detail in Section 3.2 and aspects of response rate in Section 3.3. Three participants were later excluded from analysis, due to concerns about pre-exisiting conditions which might have confounded the results. The study group of 76 therefore included; 23 subjects selected from those who on the basis of the results of the field studies were considered to have definite or probable neurological abnormality (well-defined, pre-determined); together with 36 subjects who were considered to have a possible neurological abnormality, and 23 subjects who showed no evidence of neurological abnormality. Individuals with disease, or on medication, which might have confounded the neuropathy score were excluded from those selected for Phase 3. Based on the Phase 2 data these conditions included insulin-dependent diabetes, Menières disease, Parkinsons disease, thyrotoxicosis, or medication which may cause postural hypotension.

While these sample sizes are adequate for the assessments of peripheral neurological status, it was recognised that the statistical power for comparison of neuropsychological status and OP exposure would be weak, because of the limited size of the study groups.

While these sample sizes are adequate for the assessments of peripheral neurological status, it was recognised in the previous stage that the statistical power for comparison of neuropsychological status and OP exposure would be weak, because of the limited size of the study groups. Further stratification by exposure within case-control groups was considered. However, this would have meant early linkage of Phase 2 medical and exposure data, something we wished to avoid (see later); it would have complicated the recruitment of Phase 3 subjects; and might have little advantage, in that random sampling within Phase 2 neuropathy groups was expected to provide quite a wide range of exposures anyway. Stratification on age as an exposure surrogate was considered as a means of bypassing the issue of premature data linkage, but inspection of age and exposure data from a sample of subjects suggested no strong relationship between them. On that basis, no further stratification was attempted.

2. CLINICAL NEUROLOGICAL INVESTIGATIONS

2.1 NEUROLOGICAL ASSESSMENTS

The clinical effects which have been investigated are:

1. Large peripheral nerve fibre function (using quantitative indices),
2. Small peripheral nerve fibre function (using quantitative indices),
3. Function of neuromuscular junction (using quantitative index),
4. Neuropsychological effects assessed by a) psychomotor performance, b) learning, c) memory.

The tests have been selected to maximise the information that can be gained about involvement of different parts of the nervous system in a reasonable time. The investigations assess the nerves mediating sensation and motor activity in the limbs, including the motor and sensory functions of large fibres in the peripheral nerves, small peripheral nerve fibre functions, including myelinated (A δ) and unmyelinated (C) fibres and their receptor terminals. Therefore, the battery of the investigations was chosen to provide indices of the functional integrity of the whole peripheral nerve fibre population, and was be based upon the Mayo Clinic Methodology (Dyck *et al*, 1985).

The Mayo Clinic method includes the following battery of tests:

a. *Neurological Symptom Score* This questionnaire includes selected clinical symptoms known to occur in neuropathy, scored on the basis of present (1) and absent (0).
b. *Neurological Disability Score* This involves a clinical examination and results are scored on the basis of severity from no deficit (0) to complete loss of function (4).
c. *Quantitative Sensory Tests* This uses a Computer Assisted Sensory Examination (CASE) for the detection of thresholds for vibration, touch-pressure and temperature sensation in the great toe and foot.
d. *Nerve Conduction Studies* This uses standard techniques to measure both sensory (sensation) and motor (muscle power) nerve function in the upper and lower limbs.

2.1.1 Neurological symptoms

The Mayo Clinic neurological symptom questionnaire was originally designed as part of a battery of tests to improve the diagnosis, and estimate the prevalence and severity of polyneuropathy (disorder of function of more than one nerve) among specific sub-groups of the general population. The structure of the questionnaire is discussed in more detail in the Phase 2 report.

The questionnaire was the same as that used during the Phase 2 epidemiological survey, but excluding details of occupation or details of relevant occupational exposure. This was to ensure that the INS survey team did not have access to information about the subject which might bias their assessment of neurological status. The questionnaire was administered by a neurologist in a hospital environment, whereas in Phase 2 the questionnaire was administered by technicians in the subjects farm house or place of work. The questionnaire used in the field studies was designed to detect possible chronic neurological effects which may be associated with exposure to organophosphates. Questions therefore focused on symptoms occurring in the upper and lower limbs, and questions on cranial nerve involvement were excluded.

2.1.2 Clinical assessment

The clinical assessment is also based on the Mayo Clinic criteria. In general neurological assessment was performed on the right side of the body (ie. reflexes, sensation, muscle power) unless the neuropathy

questionnaire data for an individual suggested the existence of a right sided injury that was independent of any effects from organophosphates. Both upper and lower limbs were assessed. Muscle power was assessed by applying an inverse MRC scale, where a score of zero signifies normality and a higher score signifies increasing abnormality. Again these assessments were performed by a neurologist, and in general this would be the same doctor who had completed the neurological symptoms questionnaire, since the intention was to reach an expert diagnostic opinion based on all the clinical information, excluding occupation.

2.2 NEUROPHYSIOLOGICAL INVESTIGATIONS

2.2.1 EMG and nerve conduction studies

These studies focus on large peripheral nerve fibres. Motor nerve conduction and late response studies were carried out in both upper (motor and sensory on the right median nerve) and lower (right common peroneal motor and right sural sensory) limbs using standard techniques. EMG (electromyograph) studies included standard concentric needle studies on the right extensor digitorum brevis, tibialis anterior, and extensor digiti communis.

The EMG, using disposable concentric needle electrodes, was scored using established methods. This takes into account the degree of occurrence of spontaneous potentials at rest and polyphasia of motor unit potentials. The amplitude of potentials at maximum voluntary contraction was also noted but was not used for scoring. The outcome variables were a score for each muscle and sum of scores.

Nerve conduction studies were carried out using standard techniques (Kimura, 1989) yielding numerical values which were recorded. Motor conduction studies gave latency (delay from stimulus to the first deflection of the muscle action potential), the peak to peak muscle action potential amplitude, nerve conduction velocity and F wave (monosynaptic reflex response) latency and persistence. In the sensory studies the following parameters were measured: the peak latency (delay from stimulus to the peak of the nerve action potential), peak to peak amplitude and nerve conduction velocity (distance between the stimulation and the recording sites divided by the peak latency)

2.2.2 Single fibre EMG (jitter measurement)

Single fibre EMG studies were performed on the right extensor digitorum communis muscle to investigate the integrity of the neuro -muscular junction and allow fibre density assessment of the motor unit. Jitter with block and assessment of concomitant block was also performed.

In the analysis measurements from ten different motor units are combined. The mean jitter from the ten measurements is recorded (the mean interval between the stimulus and muscle fibre response for 100 stimuli), as is the percentage of abnormal jitter, and the mean percentage of blockings (the absence of a muscle fibre response to a nerve impulse).

2.2.3 Quantitative Sensory Tests

Quantitative sensory testing (QST) was undertaken by measuring hot and cold thermal thresholds on the dorsum of the right foot to test for small peripheral nerve fibre function and vibration threshold over the middle of right index metacarpal and 1st metatarsal bone to test the large peripheral nerve fibre function. The hot threshold (HT) tests the unmyelinated C fibres; the cold threshold (CT) tests the thinly myelinated A delta fibres while the vibration threshold tests the thickly myelinated A beta fibres. The equipment used was the same as in the Phase 2 field studies, with the addition of the Glasgow Vibration System (GVS). Further details of test procedure and the physiological basis for the tests can be found

in the Phase 2 report, including the Up-Down-Transform-Rule (UDTR), which forms the basis of the QST measurement strategy.

The definition of what is abnormal for each of the tests was based on data collected from 320 normal individuals in a clinic setting (Jamal, 1986). A discussion of the choice of percentiles for defining abnormal tests and graphs of threshold limit values against age can also be found in the Appendix 4.

2.2.4 Number of changes in direction for calculating thermal thresholds.

For QST, a change in direction of two of the UDTR for calculating the thermal sensation thresholds (both hot and cold) was used in the field study while a change in direction of four was used in the clinic. In theory a better determination of the threshold should be obtained for a higher value of changes in direction. However, this means the test lasts longer and the patient might lose concentration and not perform so well.

As part of the training of the field officers in the use of the QST equipment, they performed a number of tests on colleagues under conditions that were similar to that of a hospital environment. Threshold values for both two and four changes in direction could be obtained from this pilot study. The mean differences between the results using either two or four changes in direction were 0.018 °C for hot threshold and 0.012 °C for cold threshold. This shows that two changes is as accurate as four changes of direction and that the difference in thermal thresholds between field and hospital study is not due to this factor.

2.3 NEUROPSYCHOLOGICAL INVESTIGATIONS

A battery of psychometric tests was performed and assessed the following functions; General Intelligence; Psychomotor Function; Attention ; Memory; Mood and Affect. The total time for each assessment would be about two to two and a half hours.

2.3.1 Control variable

An estimate of general intelligence, Verbal IQ, was used as a control variable, and was measured using the National Adult Reading Test. The subject is required to read a list of 50 irregular words, and the score can be converted to IQ by a standardised method.

Verbal IQ is relatively insensitive to neurological insults, but closely related to educational level, and is correlated with other functions such as information processing and attention span. It is therefore important to measure Verbal IQ, not as an index of acquired impairment, since it is most unlikely to be affected, but as a covariate to be adjusted for in the analysis.

2.3.2 CANTAB battery (Cambridge Neuropsychological Test automated Battery)

The CANTAB battery was developed for the assessment of cognitive defects in humans with degenerative brain disease. The test battery is produced by Cambridge Cognition, Waterbeach, Cambridge. It consists of a series of inter-related computerised tests of memory, attention and higher brain function and is administered via a touch sensitive screen. It is portable and allows complex tasks to be broken down into their cognitive components. The non-verbal nature of the tests makes them largely independent of language or cultural effects and a consistent mode of presentation with standardised feedback ensures high subject compliance.

CANTAB has been standardised on a large predominantly elderly population, and validated in neurosurgical patients and those with Parkinsons disease (Owen *et al.* 1993); Alzheimer's disease

(Sahgal *et al.* 1991); depression (Beats *et al.* 1995). It has also been used to evaluate early asymptomatic Huntington's disease, which illustrates the usefulness of the battery in early identification of progressive neurological disorders. The tests have application in toxicological assessments on the basis of studies of dementia from acquired disorders such as alcoholism (Joyce *et al.* 1991) and HIV infection (Sahakian *et al.* 1995). Standardised data are therefore available on normal volunteers for all the tests (Robbins *et al.* 1994).

CANTAB comprises batteries of tests, each addressing a specific area of cognition. For the purpose of this study the test areas which were selected are as follows:

Attention

I. *Motor Screening* Time to touch a cross appearing on computer screen (in msec).
ii. *Reaction Time* Five tests all involve time to release/touch pad, or make choice of items on screen. (Reaction or movement or decision time in msec).
iii. *Matching To Sample Visual Search* Choice of abstract patterns to match one presented on screen. (Total correct out of a number of trials, and response latency in msec)

Memory

iv. *Pattern Recognition* Identify pattern out of pair on screen which matches pattern shown previously. (Total correct out of a number of trials, and response latency in msec)
v. *Spatial Recognition* Identify location of white square on screen which matches location shown previously. (Total correct out of a number of trials, and response latency in msec)
vi. *Paired Associate Learning* Remember pattern and its location on screen , and point to location when the correct pattern appears in the centre of the screen (Total correct out of a number of trials)
vii. *Spatial Span* Identify the order of colour change of squares shown on computer screen (length of longest sequence recalled correctly)

As noted above attention tests include measures of psychomotor function such as reaction time.

In addition the Rey Auditory Verbal Learning test was used as a test of verbal memory. The subject is required to learn a list of 15 words read by the investigator, with the score on each of 5 trials being recorded to provide a total over all 5 trials.

The tests are graded in nature to allow for a wide range of ability while avoiding ceiling effects in young, normal subjects and lower threshold effects in the impaired elderly. Standardised scores can be produced from a large pool of normative data.

A score was assigned by the investigator for each of the tests performed and entered onto a score sheet which was sent to the IOM for processing. In addition, raw data for all subjects was down loaded onto a diskette which was also available to the IOM team performing the analysis.

The rationale for the choice of these tests from the CANTAB battery can be summarised as follows . It is known that psychomotor tasks (motor screening and reaction time) are sensitive to the effect of drugs which act on the CNS, and it is reasonable to hypothesise that such tests could show an effect in the present investigation. Evidence already exists that exposure to organo-phosphates (OPs) is associated with peripheral neuropathy and so psychomotor impairment, particularly of the motor component, would be predicted.

Attention, concentration and memory are arguably the functions most sensitive to damage to the CNS from a wide range of causes. It has been reported that individuals exposed to OPs have difficulties with

memory and concentration, although this was not confirmed in a recent study. It is important to investigate these aspects of cognitive function in more detail.

Slowing, or increased error rate when carrying out tasks which require ongoing information processing (involving attention, concentration and memory)is a common consequence of diffuse brain injury. Impairment of this function in relation to exposure to OPs has been reported, but possibly only when there are clinical signs of toxicity (which may be suggested by coexisting neurophysiological abnormality).

2.3.3 Mood and Affect

Both of the survey instruments used were paper based, and administered by a psychologist.

a. *General Health Questionnaire* (Goldberg, 1979).

This standardised 28 item questionnaire was used to evaluate psychiatric morbidity. The 28 item version has four sub-scales; somatic symptoms; anxiety and insomnia; social dysfunction; and severe depression.

b. *Hospital Anxiety and Depression Scale* (Snaith, 1983)

This questionnaire assesses frequency of symptoms in relation to both anxiety and depression, with increasing frequency being assigned a higher score. The maximum score attainable for both anxiety and depression is 18.

Since the mechanism of action of OPs is to inhibit brain acetylcholinesterase there is a theoretical basis for predicting that depression could be a consequence of exposure to such substances. Depression, anxiety and sleep disturbance have all been reported in OP users. The above questionnaires assess the relevant symptoms and provide quantitative estimates of the severity of the complaints.

3. RECRUITMENT OF STUDY SAMPLE

3.1 SELECTION CRITERIA

Selection of subjects for the clinical studies was on the basis of pre-defined criteria, on the outcome of neuropathy scores derived form the responses to neuropathy questionnaire and sensory tests performed during the second phase epidemiological field studies. The criteria were based on the Mayo Clinic system (Dyck *et al*, 1985), which can be summarised as follows:

	Classification	Criteria		
1	No neuropathy	SS < 1	AND	QST score = 0
2	Possible neuropathy	SS ≥ 1	OR	QST score ≥ 1
3	Probable neuropathy	SS ≥ 1	AND	QST score ≥ 1
4	Definite neuropathy	SS ≥ 2	AND	QST score ≥ 1

SS is the symptoms score derived from the responses to the symptoms questionnaire. The QST score is the sum of the number of indicators of abnormality from the three quantitative sensory tests (QST) corresponding to hot, cold and vibration thresholds.

Phase 3 aims to refine the understanding of the nature of the neuropathy which may result from OP exposure, and so it was considered to be helpful to restrict this exercise to the sheep farming group. Also, in considering neuropsychological profiles, we were aware that certain inherent neuropsychological characteristics might influence both choice of job, and performance on specific tests which are not related to occupational exposures. It was therefore considered desirable to design out these potential occupational selection effects.

3.2 RECRUITMENT OF STUDY GROUP

The neuropathy scores from the field studies were calculated and on the basis of this information individuals, were placed into the following categories: 'probable or definite' neuropathy (48 subjects); 'possible' neuropathy (516 subjects) ; or 'no' neuropathy (125 subjects).

The exceptionally high number of 'possibles' pointed to methodological problems. It arose because of high scores for 'abnormality' in the Phase 2 QST scores, notably cold sensory threshold and, to a lesser extent, vibration threshold also. These scores in turn reflected an inconsistency between the QST field measurements, especially cold, and the clinical reference values from which 'abnormality' was defined. It was unclear if the inconsistency arose because of problems with the QST field measurements, for example, because of low ambient temperatures during the fieldwork, or because the reference values were inappropriate, or both. The issues are described and discussed in more detail in the Phase 2 report (Pilkington *et al*, 1999).

Two hundred and forty seven subjects would have been classified as possible neuropathy on the basis of the cold score alone. We did not think that it made sense to classify these as 'possible neuropathy'. Selection of 'possibles' for Phase 3 was therefore based on the remaining 269 a strategy which, we believed, would still allow a good assessment of cold threshold in the clinical studies. Eighty individuals were selected at random from this group of 269, and a further 80 from the 125 'no' neuropathy group. Due to the small numbers, all 44 sheep farmers from the 48 individuals in the merged probable/definite category were also invited to participate in the clinical studies.

Each individual was sent a letter inviting them to participate in the clinical studies in Glasgow. They were also given some indication of their results from the field study. It was anticipated that those who had normal results would be unwilling to travel and participate in the next phase of the study, although this proved not to be the case. The letters sent also gave details of the location of the clinical studies and individuals were informed that travelling and subsistence costs would be reimbursed, and any other reasonable costs associated with their visit. Each letter also contained a pre-paid reply envelope and a reply slip, on which they could indicate whether they were willing to participate in the study or not. By tracking non-response rates it was possible to determine how many letters needed to be sent out for the possible and no neuropathy category in order to ensure an adequate number of participants for the clinical studies.

3.3 TRACKING OF NON-RESPONSE

When there had been no reply to the letter of invitation to take part in the clinical studies, individuals were followed up by phone approximately two weeks after the letters were sent. As with the earlier field survey, this often resulted in a positive response when farmers had the opportunity to discuss the survey in more detail. However where farmers were unable to take time off due to limited cover for their farm duties or due to other reasons, the reason for inability to participate was recorded. A number of farmers returned the reply slip sent out with the letter of invitation stating that they were unwilling to take part in the clinical studies. This group were not followed up further.

4. METHODS

4.1 SURVEY TEAM

The team performing the neurological and neuropsychological tests were based at the Institute of Neurological Sciences (INS) in Glasgow and included Dr Goran Jamal (Consultant Clinical Neurophysiologist), Dr Stig Hansen (Principal Clinical Physicist), Dr Musa Abdel -Azis (Registrar), Dr Peter O Julu (Registrar), Dr Sami Al-Rawas (Clinical Research Fellow), Dr Ruth Gilham (Consultant Neuropsychologist), a psychology research assistant from the same department, and nursing staff who accompanied the participants throughout the assessment.

Throughout this study, care was taken to ensure that knowledge of parts of the study did not unawarely bias the conduct of later parts. The methods to control against unaware bias in Phase 2, which centred on delaying as far as practicable any linkage of exposure and medical outcome data, are described by Pilkington *et al*, 1999.

Similar arrangements were put in place for Phase 3. The nursing staff acted to ensure that information which could potentially bias the outcome of the assessment was not divulged by the participants to the clinical staff. The nursing staff were not directly employed by the INS, and received training appropriate to the survey from IOM staff.

Different individuals were appointed to perform each component of the clinical studies. Dr Al-Rawas administered the neuropathy symptoms questionnaire and carried out the clinical neurological assessment. Dr Julu performed the nerve conduction studies and Dr Abdel- Azis performed the electromyography (EMG) and single fibre EMG (SFEMG) assessments. Dr Ruth Gilham performed the neuropsychological assessments together with a psychology research assistant.

The steps taken to limit bias in data collection and processing for Phase 3 are given in discussed in Section 4.6, and further general discussion is given in Section 4.5.

4.2 PILOT STUDY

The tests which were performed during the clinical studies are commonly performed within the neurology department at the INS. However prior to the clinical studies the INS team performed a number of assessments on outpatients attending the department to assess the time taken for the series of tests to be performed and the most appropriate sequencing of the assessments. It proved that the neuropathy questionnaire together with the scoring rules gave the expected results. All patients with a generalised neuropathy were identified correctly. Patients with one-sided compression lesion were less obvious but were identified correctly (no neuropathy). The questionnaire was also able to exclude a patient with bilateral compression lesion in the upper limbs if a criterion that lower limbs should be affected first in a toxic neuropathy was introduced. It is recognised that patients with bilateral compression lesion in the lower limbs could be identified as having neuropathy but this is encountered extremely rarely.

4.3 EXPOSURES DURING INTERVAL SINCE FARM VISIT

The clinical studies were performed at a time which avoided the peak period of pesticide usage in relation to sheep dipping practices. However it was feasible for recent pesticide exposure to have occurred from other sources. A questionnaire (see Appendix 7) was designed to identify any recent

exposures to pesticides, where acute effects of significant exposures potentially could have influenced the outcome of the neurological assessments performed.

This brief questionnaire asked about a range of sheep dip related tasks, and whether and at what time these had been performed since the survey team had visited the subjects on their farm. Handling concentrate and other incidents which may have resulted in significant exposure were also included. The subjects were also asked about exposures to pesticides from other tasks on the farm or in the home, and other exposures such as solvents or vibrating equipment which may have influenced the outcome of some of the tests.

The questionnaire was administered by the nursing staff at the beginning of the assessment. Subjects were also told at this time that they must not divulge any details relevant to their job, to ensure that the survey team were not inadvertently given information concerning the likely exposure to OPs of the subjects. The nursing staff also monitored this throughout the assessments and any relevant information which was divulged was noted.

It was not intended to add the additional days of dipping to the exposure data obtained during Phase 2 as this information was principally intended to assist interpretation of the Phase 3 neurological and neurophysiological data. In particular if the results of symptoms scores or sensory tests obtained in Phase 3 differed markedly from those in Phase 2 then data on recent exposure could help to explain this variation.

4.4 SURVEY PROCEDURE

All assessments were performed between January 1997 and the end of May 1998 which avoids periods of peak pesticide usage. Based on experience from Phase 2, it was also a period when farmers were prepared to set aside time to participate in this type of study.

The INS team were responsible for completing neurological symptoms questionnaire and clinical assessment forms, and recording results of neurophysiological and neuropsychological tests, and checking the completeness of the records. The data was then transferred to the IOM in an agreed format for data processing and analysis.

The subjects who agreed to participate in the clinical studies were sent information about appointment time, travel and accommodation details, and procedure for reimbursement of expenses. The INS were informed about the appointment times and names and survey numbers of subjects attending. All participants were met at the INS by one of the nursing staff allocated to this study, and the nurse remained with them throughout the day. The assessment began by completion of the questionnaire which detailed exposure during the interval since the farm visit. The subject was then allocated to the neurological assessment or the neuropsychological assessment for the remainder of the morning session. Subjects then completed the other half of the assessment in the afternoon. It was realised that fatigue may influence the results of the neuropsychological assessment, the time of day at which the assessment took place was recorded.

The neurological assessment comprised: the modified Mayo clinic questionnaire and clinical assessment which were performed by a neurologist; the quantitative sensory tests for heat and cold and vibration threshold which in these studies were conducted by a physicist; and the additional tests of nerve conduction, electromyography (EMG) and single fibre EMG also performed by senior medical staff within the neurology department. This assessment took approximately two and a half hours to complete. The neuropsychological assessment included the interactive computer based CANTAB battery, the GHQ and HAD scale. This assessment took approximately one and a half hours to complete.

Wherever feasible the neuropathy symptoms questionnaire and the clinical assessment were performed by a different investigator than the person responsible for performing the EMG and nerve conduction studies. The EMG and nerve conduction studies in general were performed on the right side of the body only, due to the time consuming nature of these tests, and for investigation of a condition which produces bilateral effects. It was therefore necessary that the questionnaire and clinical assessment were performed first, as this determined the existence of any unilateral condition which would have rendered inappropriate an assessment of the right side of the body. The information passed to the investigator performing the EMG, nerve conduction and QST assessments was limited to the suitability of using the right side of the body. This information was documented by the research coordinator, and handed to the investigator performing the EMG and nerve conduction studies.

On completion of the assessment individuals were informed that they would receive a brief report on the outcome of their investigations once analysis of the data from the clinical studies had been completed.

4.5 QUALITY ASSURANCE

In order to ensure that the INS survey team were not given any information which might subconsciously influence their assessment of neurological status of the participants in the clinical studies, the recruitment of subjects based on neuropathy outcome from the second phase epidemiological study was performed by the IOM. The INS team were only provided with the name and survey number for the participants. At the request of the Steering Committee only the neuropathy data from the second phase study was analysed prior to the clinical studies at INS. Therefore even the IOM team had no knowledge of any trends within the exposure data at the time of the clinical studies.

As stated earlier independent nursing staff were present throughout all the assessments at INS, and in particular were asked to ensure that subject information which might bias the outcome of the clinical assessments was not divulged to the INS survey team. The nursing staff received training in relation to the conduct of the survey and questionnaire administration prior to the survey. This training was provided by a member of the IOM team.

The INS survey team also recorded data from the additional neurological assessments performed during the clinical studies, as agreed with the Steering Committee, which would allow an independent assessment of how the results had been interpreted, if required.

4.6 DATA PROCESSING

4.6.1 Data collection

The INS were provided with a weekly list of appointments on which the subjects were identified by name, date of birth, and a study number. The study number was the randomised identity assigned to the neuropathy data in Phase 2 (N-number). The neuropathy score which had formed the basis of selection was not disclosed to the INS.

At the end of each week the forms completed in the INS clinic from that week's subjects were sent to the IOM by one of the independent nursing staff. That is:

Neuropathy Questionnaire (phase 3)
Sensory Testing Record Sheet (phase 3)
Neuropathy (Signs) Record Sheet
Nerve Conduction & EMG Record Sheet
Neuropsychology Data Recording Form

The 'interval since farm visit' questionnaires were administered by the nurse and was not disclosed in the clinical examination. They were stored separately and returned by the research coordinator to the IOM with the rest of the neurology forms at the end of each week. Copies of the forms were kept at the INS to safeguard against loss in transit to the IOM. The neuropsychology forms were returned separately by the neuropsychology assistant, when preliminary data scoring had been completed.

On receipt by the IOM the two sets of forms (neuropsychological and neurophysiological data) were controlled to ensure that a subject's forms were complete, and that the name and date of birth given by the subject at the clinic matched as expected with the identifying data from Phase 2.

4.6.2 Key entry and validation

The data from the forms was key-entered onto computer using KEIII software. The study identifier (N-number) was keyed along with subject's identifying details from the neuropathy questionnaire, the subject's responses and those Phase 3 QST measurements that would be comparable to Phase 2. The 'interval since last visit' data was also keyed. The key-entry software had been programmed to provide checks on valid responses to the questionnaires and valid ranges of measurements.

Any inconsistency between the recorded data and the valid responses was referred to the systems analyst who determined with the Project Leader an appropriate value to impute for these cases. This value was recorded on the form but distinguished from the original. Usually the attribution was to treat the doubtful data as missing (which was recorded by a special distinguishable value),

For completeness the other clinical data, not comparable with Phase 2, was also keyed. Reconciliation of this data to expected values was not carried out at the IOM. Instead a copy of the keyed data was loaded onto a spreadsheet and returned to INS.

4.6.3 Database and retrieval of information

As described in phase ii a database was designed and constructed to maintain the data for this project using SIRpc version 3.2 (SIR,1993) on a PC. A hierarchical design was used to facilitate the retrieval of information about individuals. The records for Phase 3 had the same form as those for the comparable measurements in Phase 2 but were distinguishable. Clinical data relevant to comparability of Phase 2 and Phase 3 were loaded onto the database. However, other clinical data specific to phase 3 were not included.

SIR retrievals were written to derive the symptoms scores, QST scores and neuropathy outcome scores according to the scheme described in 3.1 above. This is identical to the scoring scheme defined for Phase 2, and was implemented by the same database retrieval method as in Phase 2 but operating on the Phase 3 records. The QST score depends on comparison with an age dependent threshold. The age used was that at the time of clinical examination. The scoring of the neuropathy questionnaire is not age dependent. Care was taken with missing data to avoid the generation of a false positive score for the section in which the data was missing.

The clinical data had been loaded to the database using the pseudonym-numbers for neuropathy in Phase 2 (N_number) which was also the Phase 3 study identifier. At this stage the exposure history data from phase 2 was still indexed differently (by H_number) . This meant that individuals existed as two cases on the database but with no possibility at this stage of matching medical with exposure information. Thus descriptive statistics and comparisons of phase 2 and 3 data could be carried out without any results being influenced by knowledge of the individual's exposure. Data forwarded for statistical description used the neuropathy pseudonym identifiers for the clinical data and not the names of individuals or farms.

The Phase 3 scoring was also sent to INS to permit the derivation of classification profiles for other clinical data , and for neuropsychology. These were carried out at INS in accordance with agreed principles.

Only after all validation and scoring was completed, both of neuropathy and exposure data, was the project team provided with the data which matched the history of occupational exposure (H_number) to neurological data (N_number).

4.6.4 Data protection and Confidentiality

The database is protected by a password, and by further access-control words which prevent alteration of the data except by the project analyst/programmer (and his line manager). These also limit the reading of data to project team members on a need- to-know basis. In particular the data derived for statistical description and modelling has never disclosed the names of the subjects.

The database has been backed-up to off-line tape media.

4.6.5 Data Archiving

At the completion of the project the back-up media of the database, derived files for statistical analysis etc, the data keyed and copied to INS and manuscript records on which the IOM has collected data from farmers (or other workers) will be archived and retained. The raw data held by INS will be archived by them and retained.

5. RELIABILITY OF FIELD MEDICAL MEASUREMENTS

5.1 STATISTICAL METHODS

The aim of this component of the clinical study was to establish whether the classification of subjects using the modified Mayo method based on field measurements was comparable to the more thorough and controlled examination in the clinical setting.

Since classification variables were categorical, reproducibility was assessed by cross-tabulation by the two classifications and summarised using the kappa statistic (Armitage and Berry, 1994). The kappa statistic is a means of quantifying the degree of agreement relative to that expected by chance (κ=0), if the two classifications were independent, and perfect agreement (κ=1), when all subjects lie on the main diagonal of the two-way table. It is to be expected that even by chance some subjects will lie on the diagonal. It is possible that agreement could be worse than chance, i.e. where some sort of aversion is operating between the two classifications, corresponding to negative kappa statistics bounded by κ=-1.

When classifications are ordinal, i.e. the categories can be ranked, as here, then it is possible to use a weighted kappa statistic where, for example, a difference in agreement of only one category is given less weight that a difference or two categories, and so on. As with all one-dimensional summary statistics, however, kappa does not give a complete picture of agreement, being conditional on both marginal distributions, and not invariant to changes in the number of categories used. It is also difficult to determine the precise meaning of absolute values of kappa in terms of what constitutes good and bad agreement. Fleiss (1981) suggests that values higher than 0.75 represents very good agreement, while values lower than 0.4 represent poor reproducibility. In this study emphasis was placed less on absolute levels of agreement and more on the relative performance of several classifications made on the same subjects for which it is ably suited. Statistical analysis was carried out using the Genstat statistical software package (Genstat, 1993).

The level of agreement between the continuous QST threshold measurements was assessed informally using scatter plots. The level of agreement was quantified by analysing the distribution of the individual field versus clinic differences, summarised by the mean and variance.

5.2 COMPARISON OF FIELD AND CLINICAL MEDICAL OUTCOMES

5.2.1 Classification of neuropathy

The comparison of the field and clinical classification of neuropathy using the original (Section 3.3) neuropathy scoring system is shown in Table 5.1, which is based on all 79 subjects who attended the clinic as part of Phase 3. In all, 40 (51%) of the 79 subjects were classified in the same category on both occasions. There was no strong evidence of bias, with 18 (46%) of the 39 off-diagonal elements classified higher in the clinic than in the field, and 21 lower. Overall agreement was however only modestly better than chance, as measured by the kappa statistic (κ=0.26, SE 0.08). A weighted kappa, taking account of the number of categories of difference, was similar at 0.27, suggesting that where differences occurred, they were not necessarily simple movements into the next highest or lowest category. Since the subjects included in the clinical comparison did not include any of those within the field study group with a unsubstantiated positive cold QST outcome, it may be that this measure of agreement has over-estimated reproducibility in the field study group as a whole. The disagreement is noteworthy, for example, in that only 8 (36%) of the 22 subjects classified as 'no neuropathy' based on measurements in the field were again classified as 'no neuropathy' when the same scoring system was applied to measurements made in the clinic. In total, 11% of individuals were classified at opposite ends of the neuropathy scale (e.g none/probable, probable/none) on each occasion.

5.2.2 Agreement of component scores

The reproducibility of each of the four component scores that were combined within the neuropathy scoring system was also investigated in an attempt to highlight any particular principal source for the lack of agreement overall. Comparisons between the field and clinic symptom score and the three QST outcomes are shown in Tables 5.2 to 5.5, respectively.

Exact agreement for the grouped symptom score was found for 51(65%) of the 79 subjects (Table 5.2) with no evidence of bias, the 28 off-diagonal elements being were distributed equally between those with higher scores in the field and those with lower. Overall agreement was significantly better than chance, based on a chi-square test of association. The kappa statistic, κ=0.37 (SE 0.10), suggested reasonable reproducibility of the symptoms questionnaire between the field and clinical studies, while the higher value of the weighted kappa of 0.46, suggested that often disagreement was only by one, rather than two, categories. Table 5.2 shows that 35 (78%) of the 45 subjects with lowest score in the field again had lowest score in the clinic, with similarly high reproducibility of the highest score (13 from 17, or 76%).

Among the three QST outcomes (Tables 5.3-5.5) , the proportion on the main diagonal, designating exact agreement, ranged from 60-68%; and this was significantly better than chance for the heat and vibration tests but not the cold test. This corroborated earlier evidence, among ceramics workers, of a lack of comparability between the cold threshold measurements in the field and the clinical reference values. Relative to chance, overall agreement was better for the vibration test (κ=0.30) than for either the hot (κ=0.22) or cold tests (κ=0.18), but, in all three, was poorer than for the symptoms score.

5.2.3 Agreement of measured QST thresholds

The actual measured thresholds for each of the three QSTs were compared between the field and clinic and the results are presented as scatter plots in Figure 5.1. These show Phase II versus Phase III logarithmic values of hot, cold and vibration thresholds for the 79 subjects that attended the clinic. The positive correlation for each threshold indicate that a patient with a high threshold in the clinic is most likely also to have had a high threshold in the field. Adverse conditions (low temperature) are thought to be the cause of the bias in the field measurement. The use of normative values from the clinic for the field study measurement has been a significant factor in the difference in classification between field and hospital study.

Since inter-individual variation in thresholds tended to increase with the mean threshold, all three thresholds were analysed on the logarithmic scale. These show a significant degree of linear correlation between the field and clinic measurements. Correlation coefficients were 0.71, 0.44 and 0.66, for the hot, cold and vibration measurements respectively. There was evidence of a bias in the hot and vibration measurements. This is summarised in Table 5.6. For the hot QST, thresholds tended to be proportionally lower, by a factor of two, in the field compared to the clinic, although from figure 5.1(a), there was evidence that the bias was greatest for those with the lowest thresholds. For the vibration QST, field thresholds were proportionally higher than clinical thresholds, again by a factor of two. Adjusted for these biases, within individual field-clinic differences varied by, on average, a factor of approximately 2.7, based on the geometric standard deviations (GSD).

Even thresholds measured in the clinic are prone to random measurement error which is exhibited as intra-individual variation in repeated testing of the same subjects. However, if we were to assume that measurements in the clinic in some way represent 'truth', and that the field measurements on the same individual consisted of this truth plus added noise due to poor reproducibility specifically in the field, we would expect to see much greater inter-individual variation in the field than in the clinic, if the same group of individuals were tested in both settings. Comparing the inter-individual variances of the phase 3 subjects as presented in Table 5.6, the ratios of the geometric standard deviations (field v. clinic) were

1.25, 0.99, 0.96 for hot, cold and vibration thresholds respectively. This indicated that inter-individual variation was only slightly higher in the field compared to the clinic for the hot test, but comparable for the other two tests.

5.3 OUTCOMES OF THE COMPARISON OF FIELD AND CLINICAL MEDICAL MEASUREMENTS

5.3.1 Choice of neurological response variables

The Mayo Clinic methodology for the diagnosis of neuropathy has only ever been validated in a clinical setting using professional clinic staff. In a study of this scale, the only practical option was to carry out the various tests and procedures in the field using trained technicians. It was expected that there would be less than perfect agreement between measurements made on the same individuals in the field study and in the clinical study. Although using the chosen method there was clear evidence of association between the classifications of neuropathy made in the two settings, the level of agreement was only modestly better than chance, and resulted in a substantial minority of subjects (11%) being classified at opposite ends of the classification scale on each occasion. Therefore, it was decided that the cross-sectional study of exposure-response relationships would *not* use the modified version of the Mayo Clinic neuropathy scoring system as described in section 3.3. Instead, it was decided to use four distinct neurological response variables, the symptom score and the three continuous QST threshold measurements, in the analysis of exposure-response relationships in the field study data

The symptom score had proved reasonably reproducible and it would be used as a simple indicator (<1 or ≥ 1) of the presence or absence of reported symptoms using the scoring rules described earlier.

Although, in comparison with clinical reference values, the classification of subjects into 'normal' and 'abnormal' groups based on the QST thresholds had proved less reproducible, there was still benefit in using the actual measured thresholds as individual continuous response variables. This is because, if a continuous measurement of a physical quantity, such as a sensory threshold, does in fact correlate with the true level of underlying damage, then there is a substantial loss of information if that variable is instead used to dichotomise the response into binary high/low categories based on comparison with a fixed external reference line. This is most clearly demonstrated using two individuals who, although they have almost identical threshold measurements, fall marginally on either side of the reference line and are therefore categorised differently. This will be compounded by the presence of non-negligible intra-individual variation, as exists for sensory thresholds. This point is particularly pertinent in the current study, where the hypothesis under investigation is that chronic low-level exposure leads to incremental neurological damage. Detection of incremental damage to the nervous system would best be served by retaining the sensory test thresholds on their original, continuous scale.

5.3.2 Implications for the study of exposure-response relationships

The main implication for using four separate neurological response variables was that it required four separate exposure-response regression analyses. Although unifying the results of a number of exposure-response relationships can be sometimes prove difficult, the use of four response variables can be viewed as a benefit in that different manifestations of neurological disease can be identified and related to exposure variables.

Biases were observed in the field QST thresholds in relation to those measured in the clinic. There was evidence that this was due to lack of control of the core temperatures of the farmers who were principally surveyed during winter months. Comparison of the field and clinic QST thresholds resulted were consistent with evidence for biases due to low core temperatures. In cold temperatures, increased sensitivity to heat might be expected to reduce hot thresholds, resulting in a bias downwards in the field

compared to the clinic. Equally, cold temperatures would reduce sensitivity to both cold and vibration sensations both of which were higher on average in the field compared to the clinic.

However, in a linear regression framework, a bias in the sensory test thresholds, that applied independently of exposure, would not effect the detection of a statistical exposure-response gradient whether one truly existed or not. Random measurement error is an unavoidable component of any measurement system, and in this context, would apply in a clinical setting as well as in the field. In fact, as noted above, there was no evidence that the field measurements incorporated a significant component of *additional* random error relative to the clinical measurements. Therefore, the random scatter in the plots of field versus clinic QST thresholds is likely in the main part, to reflect inherent measurement error in sensory tests of this type. The presence of random error such as this in response variables does not result in biased estimates under a regression framework, but only serves to weaken the power of the analysis to detect an exposure-response gradient where one truly exists.

5.3.3 Further analyses of field and clinic reproducibility

The report of phase 2 of the current study (Pilkington et al, 1999) describes, in Chapter 8, the results of exposure-response analyses using the four neurological response variables as described above. One clear result from the analysis of symptoms prevalence was that subjects, predominantly farmers, in the English regions reported symptoms more often than subjects in the Scottish regions of the same age, sex and exposure, as quantified by an odds ratio (OR) of 2.

There was the opportunity, with the clinical data, to compare the reproducibility of symptom reporting between the Scottish and English farmers who attended the clinic. In total, 53 Scottish farmers and 26 English farmers attended the clinic. Therefore, when cross-tabulated by symptoms score (<1, ≥ 1) in the field and clinic, separately by country, the numbers in some of the cells of the tables were small, particularly among English farmers. However, there was evidence that reproducibility of symptoms among English farmers (κ=0.55; SE 0.16) was better than that of Scottish farmers (κ=0.24; SE=0.14).

Table 8.7 in the report of phase 2 of the current study shows that the crude prevalence of symptoms among Scottish farmers was 14%, while for English farmers it was 25%. To estimate hypothetical clinical prevalence rates within the field farmer group, a simple adjustment was made by applying the rates of true positives and false negatives found among farmers attending the clinic to the corresponding numbers of positives and negatives found among farmers in the field. In this way, a clinically adjusted prevalence of 29% was estimated for Scottish farmers, with English farmers unchanged at 25%.

Although this crude calculation takes no account of the age and sex of the farmers in the field relative to the clinic, it does suggest that the higher rates of symptom reporting found among English farmers in the field were more reproducible, and, by implication, more reliable, than the lower rates found among Scottish farmers. It is, however, recognised that reproducibility in itself does not guarantee that the measurements themselves necessarily reflect a medical truth. It also suggests that the observed difference in symptoms prevalence between the two countries is not due to a systematic difference in exposures to OP sheep dips, or any other type of exposure that may lead to similar symptoms. This in line with the comparison of OP sheep dip products used by subjects from the two countries (reported in Appendix 10 of the phase 2 report) which, conditional on products recalled, does not show any marked differences in the type of products used.

The finding of possible relatively depressed rates of symptom reporting among Scottish farmers does not invalidate the results of the exposure-response analyses reported in the phase 2 report. By including a term for country differences in the logistic regression models for symptoms, adjustment was made for the relatively lower rates in Scotland, allowing valid comparison of the effects of exposure and other factors both within and between the two countries.

Comparison of field and clinic reproducibility of symptoms reporting between the two sexes, found to have different prevalence rates in e-r analyses, was made difficult by the small number of females attended the clinical study (n=11). However, there was no evidence of markedly different reproducibility between the two sexes.

Comparison of the field and clinic agreement of QST thresholds among farmers in the two countries did not suggest that the biases noted earlier for the hot (lower in the field) and vibration (higher in the field) thresholds in particular differed between the two countries. In fact, these biases were found to be consistent across several other explanatory factors, notably sex (limited by the small number of females) and exposure, when crudely categorised into high and low.

6. RELATIONSHIPS WITH NEUROPHYSIOLOGICAL DATA

6.1 ANALYSIS OF NON-RESPONSE

Perhaps not surprisingly response rates were better for those individuals who had been informed that some aspects of the initial assessment performed during phase 2 were outside the expected range for their age group. For the probable/definite category 27 out of 43 subjects (63%) contacted agreed to participate in the clinical studies, and 37 out of 80 (46%) of those classified as having possible neuropathy. There was still a satisfactory response from those individuals who had no evidence of neuropathy at the initial assessment. Thirty one of the seventy eight subjects (40%) contacted agreed to participate.

As the clinical studies were conducted in Glasgow it had been assumed that travelling distance might be a factor in dissuading subjects from the Hereford and Worcester region to participate. Whilst this reason was given by a number of subjects contacted, it was not restricted to those from Hereford and Worcester and in general the response rate from this region was reasonable when comparable with that from Scotland, with several subjects expressing concern about OPs and potential health effects among their colleagues.

6.2 DESCRIPTIONS OF PARTICIPANTS

Of those invited to a clinical assessment, 79 subjects actually attended. It was later discovered that three of these subjects had failed the criteria for inclusion into the field study group due to reporting of conditions and medication in the field medical questionnaire that could potentially confound a diagnosis of neuropathy. For consistency, these subjects were also excluded from analysis of the clinical data leaving a study group of 76 individuals.

Using the same methods as used in the field, each subject was assigned a diagnostic category corresponding to the likelihood of neuropathy. Table 6.1 describes the clinical study group broken down by category. As for the selection process, and due to the small numbers, the 'probable' and 'definite' groups were combined into a single category for analysis. After exclusions, the numbers in each of the 'none', 'possible' and 'probable/definite' categories were 17, 36 and 23 respectively. There was approximately an equal proportion of females in each category, close to the overall prevalence of 13%. The 'possible' and 'probable/definite' groups were, on average, both 7 years older than the 'none' group, consistent with the greater likelihood of neuropathy in these groups. There was a wide spread of ages within each group , overall from 20 to 66 years, which can be seen in the box plots in Figure 6.1. There was very little difference in average alcohol consumption across the three groups, close to the overall average of 9.5 units of alcohol per week, although this does not reflect the wide range of consumption, from 0 to 40 units per week, within the study group as a whole.

Overall, 67% of those attending the clinic were Scottish sheep dippers. There was a higher prevalence of Scottish sheep dippers within the 'possible' group (78%) compared to the other two groups.

Only farmers with sheep dipping experience attended the clinic, the minimum number of days dipped being five days for any individual in the study group.

There appeared to be differences in average cumulative exposure across the three groups. However, it was the 'possible' group that had the highest average exposure based on the OPEXP exposure index derived in phase 2 of the study and described in Pilkington *et al* (1999). Much of the reason for this was due to the presence of a small number of very highly exposed individuals in this group, including one individual with a cumulative exposure index of 44010, based on 1350 dipping days. Figure 6.2 shows the distributions of cumulative exposure within each of the neuropathy groups. This confirms that there

was very little difference in the exposures experienced in the 'none' and 'probable/definite' groups, which was not consistent with a hypothesis of increased likelihood of neuropathy with cumulative exposure.

6.3 CLASSIFICATION OF NEUROPATHY

For the analysis of Phase 3 neurological and neurophysiological data the subjects have been divided into three different groups based on the neuropathy questionnaire results and QST thresholds from the hospital based investigation. The criteria used for groups 1, 2, 3 and 4 (no, possible, probable and definite neuropathy) are identical to those used in Phase 2, the field study. In common with analysis carried out for other Phase 3 data, groups 3 and 4 (probable and definite neuropathy) are combined.

Detection of neuropathy using the neuropathy questionnaire results and QST thresholds gives high sensitivity but may give low specificity (Jamal *et al* 1985b) and some false positive results may occur. When other neurophysiological parameters and neurological signs are considered as well, the number of subjects confirmed as having a clinically detectable neuropathy will decrease but the decision is taken with higher probability of being certain of the diagnosis.

The 95% confidence limits of normality for individual parameters have in some cases been changed from the values originally stated. This only affects nerve conduction parameters: Motor and sensory latency, NCV and potential amplitude. This was done in order to take account of recommended laboratory practice published in handbooks (Delisa *et al*, 1994) and had the effect of setting more severe criteria thus increasing the specificity.

Three individuals had the following conditions recorded on their Phase 2 neuropathy questionnaires: rheumatoid arthritis, family history of high arches, and hypertension requiring medication which was associated with hypotension. It was decided to err on the side of caution and exclude them from Phase 3 to limit possible confounding effect.

A further four subjects were found to have a distribution of abnormalities of their neurophysiological parameters not consistent with a generalised neuropathy. Three had carpal tunnel syndrome and one subject had changes consistent with (possible) radiculopathy. These subjects, two each from of the no neuropathy and possible neuropathy groups, were excluded from further analysis.

Decision criteria for overall abnormality are described and these are based on the clinical experience that a toxic neuropathy is likely to affect the distal part of the lower limb first. Table 6.2 gives an overview over the number (and percentage) of subjects in each group who had a deficit.

6.4 MAIN RESULTS

6.4.1 Neurological signs

Cranial signs

No subject in any group had abnormal cranial signs indicating that there is no involvement of the cranial nerves.

Reflexes

Each subject was scored as having abnormal reflexes if:
the ankle reflex was absent **or**
the ankle reflex was reduced and any other distal reflex was reduced or absent bilaterally.

Sensory signs

Each subject was scored as having abnormal sensory signs if two or more modalities of sensation were affected in the classical stocking-glove pattern.

Muscle power

Each subject was scored as having abnormal muscle power if there was any weakness in the distal part of the lower limb.

The outcome for neurological signs is shown in Table 6.2. Each subject was considered to have significant signs if at least two out of the three signs (reflexes, sensory and muscle power) were abnormal.

6.4.2 Nerve conduction

Sural nerve - sensory

The sensory function in the lower limb for each subject was scored abnormal if both sural potential latency and amplitude were abnormal.

Common peroneal nerve (CPN) - motor

The motor function in the lower limb for each subject was scored abnormal if at least two out of the three action potential parameters (latency, amplitude or conduction velocity) abnormal.

The outcome for nerve conduction is shown in Table 6.2. Each subject were considered to have significant nerve conduction deficit if either sensory function or motor function in the lower limb was abnormal. This abnormality would be compatible with a toxic neuropathy.

6.4.3 Combination of neurological signs and nerve conduction

The number of subjects in each group with either neurological signs or abnormal nerve conduction are given in Table 6.2. These subjects are considered to have neuropathy. (Any patient coming through the clinic with similar findings would *be* reported to the referring physician as having a neuropathy). There is no overlap between subjects that have neurological signs and abnormal nerve conduction with the criteria used. This does not mean that these deficits do not coexist, only that the criteria for the analysis has been set rather strict. Out of 16 subjects with abnormal nerve conduction six (38%) have one neurological sign

6.4.4 Electromyography (EMG)

EMG recordings were scored according to predefined criteria. Table 6.2 shows the number of subjects in each group with abnormal findings in the extensor digitorum brevis (EDB) muscle. Four subjects had declined this investigation and the number in each group has been reduced accordingly. Most of the

abnormal scores were 1 and only one subject had a score of 2. No fibrillation or other spontaneous activity was found in any of the recordings and the abnormalities point to a remodelling of the motor unit indicating an axnonal type neuropathy with chronic changes.

6.4.5 Single fibre EMG (SFEMG)

Six subjects had SFEMG abnormalities with five of those in the possible neuropathy group. None of the individuals classified as normal on symptoms and QST had abnormal SFEMG. Of those with SFEMG abnormalities, three had one or more signs and four also had abnormal EMG.

6.4.6 Sensory versus motor abnormalities

Fifteen subjects had neurophysiological sensory abnormalities defined as abnormal sural conduction and one or more QST values. In contrast, only two subjects had abnormal motor nerve conduction and both were in the definite neuropathy group.

In general there was a trend towards increasing likelihood of an abnormal QST result with progression across the neuropathy groups from no neuropathy, to possible and probable neuropathy.

6.4.7 Small versus large nerve fibre abnormalities

A larger proportion of subjects had abnormal small nerve fibre function (47 – 65% of 72), assessed by hot or cold sensation threshold, than had abnormal large fibre function (15 – 21% of 72), assessed by vibration threshold or sural nerve function.

6.5 VALIDATION OF SYMPTOMS AND QST IN SCREENING FOR NEUROPATHY

The percentage of abnormalities of all parameter listed in Table 6.2 increases from the no neuropathy group through to the probable/definite neuropathy group. This is predictable and justifies the finding from the neuropathy questionnaire and QST measurement. The more symptoms the more likely one is to find further evidence of neuropathy.

In farmers generally, the estimated overall rates of confirmed (clinical) neuropathy is 18% when Phase 3 findings are reflected back into the Phase 2 population (See Table 6.3).

6.6 CHARACTERISATION OF NEUROLOGICAL/NEUROPHYSIOLOGICAL DEFICIT

The neuropathy found in this study is different from the pattern of neuropathy seen in OPIDN. There is a predominant involvement of motor fibres in OPIDN thus presenting with paralysis and weakness as the main feature although sensory fibres can also be affected.

In contrast, the neuropathy described here is predominantly of a sensory type both symptomatically and neurophysiologically and is characteristic of distal, chronic axonopathy with no acute features (See Tables 6.4 and 6.5). Small fibre populations are affected more than large fibre populations.

Autonomic nervous system symptoms were commonly reported by the no neuropathy and possible neuropathy groups, but were less commonly by the probable neuropathy group.

7. RELATIONSHIPS WITH NEUROPSYCHOLOGICAL DATA

7.1 STRATEGY AND STATISTICAL METHODS

All subjects who attended the clinic were invited to take part in the neuropsychological assessment. This assessment was carried out by different staff from those involved in the neurological examination and tests were carried out blind of the earlier results, including the field study.

The first aim of this component of the clinical study was to identify meaningful patterns of differences between sheep dippers with and without a diagnosis of neuropathy, in terms of the subject=s neuropsychological responses.

Table 7.1 lists and describes the test variables analysed and the codes used for later presentation. The neuropsychological variables that were recorded were grouped into three broad categories which describe different facets of an individual's functioning: psychological symptoms (mood and affect), processing time and memory/attention. In many of these tests, reaction time is defined as the sum of decision time (time to move hand from button) plus movement time (time between lifting hand from button and touching the screen. Therefore decision time is a purely cognitive event, while movement time has a greater motor component. The motor screening test was omitted from analysis because it was used as a practice task to orientate the subject to the equipment.

Although subjects were selected for the clinic on the basis of their field classification of neuropathy, subjects were grouped into three categories for comparison (none, possible, probable/definite) based on their clinical classification of neuropathy since this was expected to be a more accurate representation of their true disease status.

Each neuropsychological variable was compared across these groups in turn after adjustment for relevant confounders such as age, verbal IQ and alcohol consumption using multiple linear regression. Adjusted means were produced to test specific contrasts and, due to the large number of potential contrasts, a Bonferonni correction was applied to give an overall significance level of 5%.

Some of the variables were recorded in the form of the number of correct responses out of a fixed number of trials. As appropriate, these variables were analysed assuming binomial variation using logistic regression.

The second aim of this component of the study was to look for a direct relationship between impaired neuropsychological test outcomes and cumulative exposure to OPs. Since the clinical study group was non-random sample of the wider field study group, relationships were investigated within each of the neuropathy groups used for selection first, before pooling estimates across groups, where appropriate. This was because the clinical study group was not representative of the field study group as a whole, but consisted of a stratified sample, based on the original field classification of neuropathy, with unequal sampling fractions within each of the strata. For example, the clinical sample consisted of a far greater proportion of 'probable/definite' neuropathies, based on field classification but also, by association, based on clinical classification, compared to the field study group. Therefore, although samples could be viewed as representative *within* each field neuropathy category used for selection, allowing relationships to be analysed category by category, disregarding the stratified nature of the sample would result in possible spurious correlations, and distorted exposure-response gradients.

Each of the neuropsychological test variables were regressed against the log transformed cumulative exposure index, OPEXP, described in the phase 2 report (Pilkington et al, 1999) simultaneously adjusting for age and verbal IQ using multiple linear regression for the normal responses and logistic regression for the binomial responses as in section 7.2.

Since the GHQ (General Health Questionnaire) variables were highly correlated with the total score (GHQ_TOT) only this variable was included to reduce the number of variables for analysis. Equally, since reaction time RT_2 and RT_4 had been shown to be highly correlated with RT_5, only the latter was included from among the reaction time variables. Since the psychological symptom scale variables had not, on the whole, been related to either age or IQ, no adjustment was carried for variables when analysing GHQ_TOT, HAD_A and HAD_D (Hospital Anxiety and Depression Scales). The use of the log-transformed cumulative exposure both precluded the need to investigate the effect of potential influential values with very high exposure, and, since this was an exploratory analysis, would exaggerate the gradient of any effect making any true gradients, in particular trends less marked than a simple linear relationship, more easy to detect.

7.2 RESULTS

7.2.1 Study Sample

Of the 79 subjects attending the clinic, all except two completed the neuropsychological assessment. These two individuals, both males, aged 22 and 63 years, were uncooperative and the assessment was terminated before completion. A stated earlier, a further three subjects were excluded from statistical analysis since they had also failed the criteria for inclusion into the field study group. Therefore, the study group available for analysing neuropsychological differences numbered 74 subjects, comprising 17, 35 and 22 from the 'none', 'possible' and 'probable/definite' clinical neuropathy categories respectively.

As described in chapter six, the 'possible' and 'probable/definite' groups were on average older than the 'no' neuropathy group, but alcohol consumption was similar. Verbal IQ, measured using the NART (National Adult Reading Test) score, was likely to be associated with many of the neuropsychological tests. However, there were no significant differences in IQ between the three neuropathy groups, with mean scores close to the overall mean of 108.2 (SD 8.9). IQ scores ranged from a minimum of 88 to a maximum of 124 across the study group.

7.2.2 Psychological Symptom Rating Scales

Table 7.2 shows the results of comparing the three neuropathy groups using each variable in turn, after adjustment for age (linear term) and verbal IQ (NART: linear term). Alcohol consumption (units per week) was not positively associated with any variable and was not included as a covariate. All variables except GHQ_SDP resulted in significant differences among the three groups. Note the GHQ variables were highly discrete scores and therefore inference using ANOVA, based on assumptions of normality will be approximate. The table also shows the means adjusted to give predicted values corresponding to mean age (44.6 years) and mean IQ (NART= 108.2). In all cases, the rank order of the adjusted means was the same: 'probable' higher than 'possible' which in turn was higher than 'none'. The 'probable' group was consistently significantly higher than the other two, after using a Bonferonni correction for an overall significance level of 5%. High scores for all these variables correspond to poorer general mental health, and the adjusted mean for the 'probable' group, close to a value of 8, would be considered clinically significant.

Table 7.3 shows partial correlations among all the symptom variables using the residuals after adjusting for age, IQ and group differences. This shows some moderately high positive correlations among many of the variables. GHQ_TOT equals the sum of the four GHQ sub scales, and this explains the high correlation between this variable and the four sub scale variables. The four GHQ sub scales are S (Somatic), AI (Anxiety and Insomnia), SDP (Severe Depression), and SDY (Social Dysfunction).

The differences between groups were tested multivariately using MANOVA, allowing for the non-zero correlations among them. Although age was not a significant covariate in any of the univariate analyses,

the multivariate test was borderline (Wilk test: P=0.076). However, age was not a consistent effect, being negative related to GHQ_S, GHQ_AI and HAD_A variables, and positively related to the remainder. The multivariate test for IQ was not significant. The multivariate test of group mean differences was highly significant (Wilk=s test: P<0.001) confirming the collective strength of evidence in the univariate results.

7.2.3 Processing Time

Table 7.4 shows the results of comparing the three neuropathy groups using each variable in turn, after adjustment for age (linear term) and IQ (NART: linear term). Alcohol consumption (units per week) was not positively associated with any variable and was not included as a covariate. All processing time test variables were analysed on the logarithmic scale to stabilise the variance in the residuals. Of the reaction time variables, only RT_3 was significant at 5%, although two other variables, RT_2 and RT_5, had P-values close to this level. Of the visual search tests, PR_RT (Pattern recogniton-reaction time) and SR_RT (Spatial recognition-reaction time) were statistically significant at the 5% level.

Table 7.4 also shows the group means adjusted to give predicted values corresponding to mean age and IQ. For PR_RT and the three reaction time tests which were at least close to statistical significance, the 'probable' group mean was the highest, followed by the 'none' group then the 'possible' group. Only for RT_2 was the 'probable' group significantly higher than any other group, which in that case was the 'possible' group.

Although SR_RT was significantly different among the groups, in fact it was the 'none' group that had the highest score. Both the 'none' and 'probable' groups were significantly higher than the 'possible' group.

Table 7.5 shows partial correlations among all the processing time variables using the residuals after adjusting for age, IQ and group differences. This reveals some high correlations among the residuals, particularly among the five reaction time tests.

The differences among groups were tested multivariately, firstly using all reaction time variables then using the visual recognition latency variables, to allow for the non-zero correlation among them. For both the reaction time tests and the visual search test, both age and IQ were highly significant predictors of processing speed. In the univariate analyses, age was consistently positively related, and IQ was consistently negatively related to processing time. However, differences among the three neuropathy groups were non-significant for reaction time tests (P=0.30), and borderline for visual search tests (P=0.075), when tested multivariately.

7.2.4 Memory

Table 7.6 shows the results of comparing the three groups for each variable in turn, after adjustment for age (linear term) and IQ (NART: linear term). Alcohol consumption (units per week) was not positively associated with any variable and was not included as a covariate. Four of the memory test variables, VS_TC (Visual search- Total correct), PR_TC (Pattern recognition-TC), SR_TC (Spatial recognition TC) and PAL_TC (Paired associate learning TC) were recorded in the form of number of correct responses within a fixed number of trials, and therefore were analysed assuming binomial variation using logistic regression. The remaining two variables, SPAN (Spatial span) and RAVL (Rey Auditory Verbal learning), were analysed using multiple linear regression. Of all the memory test variables, only one, PAL_TC showed significant differences among the groups. The 'probable' group scored higher than the 'possible' group and significantly higher than the 'none' group. This indicated an improved performance in the 'probable' group, the opposite to what might have been expected had neurological damage lead to impaired neuropsychological outcomes.

There were generally weaker, and often negligible, correlations among the memory test residuals compared to among the symptoms variables and processing time variables (Table 7.7).

IQ was significantly positively associated with all the memory test variables univariately, apart from VS_TC, suggesting improved performance with IQ. Age was always negatively related to memory test variables, suggesting impaired performance with age. Age gradients were statistically significant only for VS_TC, PAL_TC and SPAN when tested univariately.

7.3 NEUROPSYCHOLOGY IN RELATION TO EXPOSURE

Figure 7.1 (a)-(q) shows plots of each of the neuropsychological variables against cumulative exposure (log transformed). Where earlier analyses had indicated that age and IQ were important predictors, the plots show residuals (observed value minus predicted value) after fitted regression effects for age and IQ (i.e.NART), in relation to exposure. The three field neuropathy categories used for selection are distinguished by the plotting symbol. Significant differences were tested first between linear trends across the three groups, using a group-exposure interaction term. If there was no indication of a significant difference, then an estimate of the slope pooled across the three groups was made. Of the 16 variables analysed, there was evidence of a difference in slopes between groups in only two: SPAN and PAL_TC. For variable SPAN (Figure 7.1 (p)), the 'probable/definite' selection group, but not the other groups, had a significant positive slope with exposure. However, this represented *improved* neuropsychological performance with exposure within this group. For variable PAL_TC (Figure 7.1 (n), the 'probable/definite' selection group, but not the other two groups, showed a significant decrease with exposure. This represents *impaired* performance within this group.

For all other 14 variables, the pooled estimate of slope was never close to being statistically significant. In 11 of these variables, the direction of the common slope (positive or negative) was in contrast to that expected under an hypothesis of impaired performance. The only three variables that were estimated to have a slope with a direction consistent with impaired performance were VS_DT (Visual search - Decision time), PR_TC and SR_TC.

7.4 SUMMARY

7.4.1 Main 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 be diagnosed as having neuropathy.

As expected, processing speed was strongly related to both age and IQ. Allowing for these effects, there was some specific evidence of slower processing times among 'probable/definite' cases of neuropathy. However, the results, across a variety of such tests, were not consistent and did not provide clear evidence of an overall slowing of processing time.

Allowing for the impairment of memory with age and for general IQ, there was no evidence of a difference in memory capability between probable cases of neuropathy and controls.

The sampling design of the clinical study was not optimum for the detection of a direct relationship between impaired neuropsychological performance and cumulative exposure to OP sheep dips. No evidence for such a relationship was found across a wide range of indicators.

8. DISCUSSION

The discussion will overview the main findings of the Phase 3 study in the light of the initial hypothesis and the reliability of the study. The relevance of the findings will be discussed in the light of current knowledge of the possible long term effects of exposure to OPs, and will consider the possible mechanisms which may have led to these findings. The potential implications of the study findings will then be considered.

8.1 REVIEWING THE OBJECTIVES

The broad aim of the study as a whole was to investigate whether cumulative exposure to sheep dip OPs is related to clinically detectable measures of polyneuropathy; and if so, to estimate the magnitude of that relationship. The specific objectives of Phase 3 were to: classify in terms of clinical disease the subjects with abnormal indices of peripheral neuropathy identified in the Phase 2 field studies; describe any associations between neurological and neuropsychological abnormalities; and to examine the relationship between neuropsychological status and estimated cumulative OP exposure.

This was achieved by a nested case-control clinical study of selected subjects with neurological abnormalities identified in the field studies, and of control subjects. This investigation aimed to provide additional information about the nature of any neuropathies found, and included a neuropsychological component which had not been practicable in the field investigation.

8.2 METHODOLOGICAL ISSUES RELATED TO DESIGN AND PARTICIPATION

8.2.1 Selection of subjects

A subset of subjects involved in the field study were invited to participate in the clinical study. To ensure a wide representation of disease status in the clinical study, the objective was to select individuals in equal numbers from the 'no', 'possible' and 'probable/definite' groups as derived from the field survey data, using the original neuropathy score. Because of small numbers, all possible participants in the probable/ definite group were invited. Random samples were selected from the 'no' and 'possible' groups, except that nobody who was categorised as 'possible' based only on abnormal cold threshold was invited. This was because of doubts about the reliability of 'abnormal' cold threshold in Phase 2. However, the subjects selected did include a wide range of cold threshold responses.

Seventy nine subjects attended the assessments at INS, and 76 were included in the study group comprising 17, 36 and 23 subjects respectively from the 'no', 'possible' and 'probable/definite' categories in the field study, as classified according to the original field study system (Pilkington *et al*, 1999) described in Section 3.3. All those who attended were sheep dippers. No ceramics workers were invited to attend the clinical study, and the few non-exposed farmers who were invited did not take part.

The 79 subjects assessed were less than had been originally intended, but this was not due to non-response, rather to unexpected delays in starting the clinical work in Phase 3. It was felt, however that sufficient subjects attended across the range of neuropathy classification to allow informative results from Phase 3.

8.2.2 Participation rates

As expected, participation rates were better for those individuals who had been informed that some aspects of the initial assessment performed during Phase 2 were outside the expected range for their age group. For the probable/definite category 27 out of 43 subjects (63%) contacted agreed to participate in the clinical studies, and 37 out of 80 (46%) of those classified as having possible neuropathy. However there was still a satisfactory response from those individuals who had no evidence of neuropathy at the initial assessment. Thirty one of the seventy eight subjects (40%) contacted agreed to participate.

It had been assumed that travelling to Glasgow (INS) for the clinical studies might be a factor in dissuading subjects from Hereford and Worcester to participate. Whilst this reason was cited by a number of subjects, it was not restricted to those from Hereford and Worcester and the response rate from this region was reasonable compared with that from Scotland.

8.2.3 Assessment of recent exposure

A questionnaire on recent exposure to OPs was administered to all Phase 3 participants by a nurse, who accompanied the subjects throughout the assessment, and remained vigilant to the need to avoid exposure information being passed onto the INS clinical team. The nurse was employed independently, and given training by a member of the IOM project team, who also screened questionnaires at intervals to ensure consistency of recording.

The format used was similar to that of the Phase 2 exposure-history questionnaire. Only one incident was recorded where the subject passed on information during the clinical assessments which might have indicated their occupational group. This information related to an electric fence, and was not thought to have allowed distinction between farming groups, and so the subject was not excluded from Phase 3.

8.2.4 Exclusions because of relevant medical history

There were three individuals who took part in the Phase 3 clinical studies who had the following conditions recorded on their Phase 2 neuropathy questionnaires: rheumatoid arthritis, family history of high arches, and hypertension requiring medication which was associated with hypotension. As these subjects had been excluded along with others with specific medical conditions from the Phase 2 analysis, it was decided to err on the side of caution and exclude them from the Phase 3 analyses of new physiology tests, and neuropsychology, to limit possible confounding. They remain however in the comparison of symptoms and QST measurements between Phase 2 and Phase 3.

In examination of the neurophysiological results of Phase 3, a further four subjects were found to have a profile of abnormalities of some of their neurophysiological parameters not consistent with a generalised neuropathy. Three had carpal tunnel syndrome and one subject had findings consistent with radiculopathy. These subjects, two each from of the "no neuropathy" and "possible neuropathy" groups, were also excluded from further analysis.

8.2.5 Steps taken to reduce bias

At the request of the Steering Committee the exposure data was not processed until the Phase 3 clinical investigations were completed. During this time the Phase 2 neuropathy scores were derived by the relevant member of the IOM team, in order to allow the selection of subjects for Phase 3. This individual was therefore blind to any data relating to exposure. More generally, to prevent the neuropathy outcome being in any way affected by knowledge of an individual's exposure the project analyst/programmer and the statisticians were kept blind to the match of identities. The matching of identities was carried out by a different analyst/programmer who had access to both sets of forms so that any apparent discrepancies of identity, arising from different transcriptions of names, were successfully resolved.

The staff at INS were only given a subject number to identify individuals for the clinical studies and had no knowledge of neuropathy classification. When the Phase 3 clinical examinations had been carried out, and the exposures calculated by the model from Phase 1, it was agreed that the exposure-response relationship could be examined. Only at this point was the matching of exposure and medical identifications carried out.

In order to limit observer bias, the different components of the neurological investigations were performed by different members of the INS team, who were blind to any previous results, and to the exposure history or field classification of the subjects being assessed. (The INS team did not know until well after the end of the study that the subjects were only farmers but thought that some would be ceramic workers.) In the case of the additional neurological tests, the data were recorded in sufficient detail to allow interpretation by independent observer. The data storage facilities for the outcomes of the neuropsychological investigations also allow a similar process to occur.

8.3 RELIABILITY OF PHASE 2 FIELD CLASSIFICATION OF NEUROPATHY

8.3.1 How reproducible are the Phase 2 field measurements and classification of neuropathy?

The symptoms questionnaire used in Phase 3 was the same as that used during Phase 2 epidemiological survey, but excluded details of occupation or details of relevant occupational exposure, and was administered by a neurologist. However, in Phase 3 the questionnaire was followed by a clinical assessment based on the Mayo Clinic criteria. The same range of sensory tests were performed in Phase 3 as in the Phase 2 studies, but were now performed by specialist neurologists under controlled temperature conditions.

The scoring system applied to the Phase 3 neuropathy data was that developed for the Phase 2 field studies and was based on the Mayo Clinic methodology. The Mayo Clinic methodology for the diagnosis of neuropathy has only ever been validated in a clinical setting using professional clinic staff. It was expected that there would be less than perfect agreement between measurements made on the same individuals in the field study and in the clinical study. There was a minimum period of 18 months between the Phase 2 field studies and the Phase 3 clinical studies, and therefore some of the variance may be explained by changes in health status during this period. The clinical studies were also performed by trained neurologists whereas, the field studies were performed by technicians. Experienced clinicians are more likely to use their own judgement in interpreting subjects' responses than technicians with limited medical knowledge, and this factor might influence the recording of symptoms. In the clinical studies it was also possible to maintain a more constant and appropriate ambient temperature, which would be expected to reduce the degree of measurement error for the sensory tests.

Whilst there was clear evidence of association between the original classifications of neuropathy made in the two settings, the level of agreement was only modestly better than chance, and resulted in 11% of subjects being classified at opposite ends of the classification scale. It was also clear that the symptoms questionnaire was markedly more reproducible than the indicators of abnormality, based on comparison with a reference population, that resulted from the three sensory tests.

In practice, differences were found between the field and the clinic, especially for QST measurements. These discrepancies are more likely explained by the difference in *conditions of measurement of thermal thresholds between hospital and field.* The measurements in the clinic was performed under ideal conditions by a highly experienced operator. The conditions prevailing during the field study were difficult often carried out in a cold room. It was impossible to maintain a reasonable limb temperature for many of the subjects and nerve function is dependent on temperature. Perception of a threshold stimulus depends on both spatial and temporal summation of nerve impulses from all the thermal

receptors being stimulated. Cooling of the nerve may affect the conduction velocity of different fibres differently causing temporal dispersion of the nerve impulses and thus a higher threshold.

Thermal sensation is also dependent on the basic skin temperature prior to stimulation. As the skin temperature falls below 28 – 30 °C, the cold threshold is known to increase whereas the hot threshold increases as the skin temperature rises above 36 – 38 °C. This could explain that a relatively higher number of abnormal cold thresholds were found in the field study compared to the hospital based study with smaller difference for the hot threshold.

On the basis of comparison of the reproducibility of Phase 2 and Phase 3 neuropathy data, it was decided that the analysis of Phase 2 exposure-response relationships would *not* use the neuropathy scoring system. The four component parts of the score were therefore analysed separately on the continuous scale in relation to exposure and not grouped by 'abnormality' according to the earlier reference values (Pilkington *et al*, 1999). The symptom score had proved reproducible and it was used as a simple indicator (<1 or ≥ 1) of the presence or absence of reported symptoms using the scoring rules described in Section 3. It was felt that the although less reproducible in the clinic, the thresholds measured in the field were still informative indicators of the relative level of peripheral nerve damage in the field study sample.

Comparison of the field and clinic QST thresholds provide evidence for the temperature effects since in cold temperatures, hot thresholds might be expected to be reduced resulting in a bias downwards as was seen in the field measurements in comparison with the clinical measurements. Equally, cold temperatures would reduce sensitivity to both cold and vibration sensations and, for the latter test, field measurements were considerably higher than those in the clinic. This comparison also suggests that the reference data used in the clinic are not appropriate for the field setting.

8.3.2 Validation and refinement of scoring system using further tests

Following exclusions, these analyses were based on 72 subjects. Twenty three (32%) out the 72 subjects had confirmation of their neuropathy by neurological signs or nerve conduction abnormality: one (7%) of the 15 subjects from the 'no neuropathy' group (the confirmed subject having abnormal nerve conduction but no clinical signs or symptoms, and no QST evidence of neuropathy); ten (29%) of the 34 individuals classified as having 'possible neuropathy'; and 12 (52%) of the 23 subjects classified as having 'probable/definite neuropathy'. These classifications are now based on symptoms and QST as measured in the clinic (Phase 3) and not as in the field (Phase2). These results confirm that the case identification methods used in the field have some degree of validity in the identification of peripheral neuropathy.

Thirteen (18%) of the 72 subjects had sensory abnormalities defined as abnormal sural conduction and one or more abnormal QST values while only two subjects (3% of 72) had abnormal motor nerve conduction and both were in the definite neuropathy group. Forty seven subjects (65% of 72) had abnormal small nerve fibre function, assessed by hot or cold sensation threshold, while only 15 (21% of 72) had abnormal large fibre function, assessed by vibration threshold or sural nerve function. Thus, small fibre dysfunction was three times more common than large fibre dysfunction. In defining the relative involvement of small versus large and sensory versus motor fibre populations, one must remember that methods used to assess them may not have identical sensitivity.

The neuropathy described here is predominantly of a sensory type both symptomatically and neurophysiologically and is characteristic of distal, chronic axonopathy with no acute features. Small fibre populations are affected more than large fibre populations.

Autonomic nervous system (ANS) symptoms were reported more commonly than peripheral nervous bsystem (PNS) symptoms in the phase 2 study. This is also the case in the phase 3 study for the "no neuropathy" and "possible neuropathy" groups but not for the "probable/definite neuropathy" group.

The results of the additional tests (clinical examination and nerve conduction) performed in Phase 3 corroborate the other aspects of the Mayo methods to detect a possibly toxic neuropathy in the clinical studies. The Mayo Clinic methodology was designed to improve the diagnosis, and estimate the prevalence and severity of polyneuropathy among specific sub-groups of the general population. As toxic neuropathy, which might occur in association with OPs, would be expected to affect the lower limbs first, this was considered within the design of the test battery. All components of the Mayo method used have been well validated (see supporting text), and were performed according to a strict protocol, and the test battery is able to give some indication of the pattern of neuropathy occurring within a population.

8.4 FINDINGS FROM BOTH PHASES 2 AND 3 REGARDING NEUROPATHY

A recent report by the joint working party of the Royal College of Physicians and the Royal College of Psychiatrists noted that the evidence that long term low dose exposure to OPs causes chronic ill health, is still the subject of much research. Studies have suggested impaired attention and reaction time (Fiedler *et al*, 1997); increased psychiatric morbidity (Stephens *et al*, 1995); minor sensory changes (Beach *et al*, 1996); and EMG abnormalities (Drenth *et al*, 1972 and Stalberg *et al*, 1978). The working party consider that the population based studies suffer from a number of methodological weaknesses, and the biological significance of some of the tests used is not clear. Written evidence received by the joint working party suggested that individuals reporting health effects were more likely to be male middle aged, and to complain of weakness, lethargy, fatiguability and be mildly depressed. In general the working party concluded that how OPs might cause these effects is still not known.

The Phase 2 field studies (Pilkington *et al*, 1999) have suggested that there was a significant difference in symptoms reporting between ceramics workers and sheep dipping farmers, which is larger than expected on the basis of age differences and only explained in part by cumulative exposure. There were similar smaller differences in cold threshold between the groups after adjustment for cumulative exposure. Sheep dippers also reported more symptoms than non-sheep dippers, and had higher cold thresholds, although these findings were not statistically significant. There were also regional differences in symptom reporting between sheep farmers in Scotland and England with English farmers reporting more symptoms. Sheep dippers who handled concentrate had three times the reported prevalence of symptoms than non-concentrate handlers.

The use of the full Mayo test battery during Phase 3, applied using recommended procedures, aimed to inform the results obtained in the Phase 2 field studies. The results of the additional tests (clinical examination and nerve conduction) corroborate the other aspects of the Mayo methods to detect a possibly toxic neuropathy in the clinic. The trend of these results across the neuropathy groupings was consistent with the broad categorisation from the Phase 2 results. Again there was a higher percentage of abnormal sensory thresholds than anticipated, including the cold threshold. Possibly the reference data used for the sensory tests included too narrow a range of individual variation.

In general the results of the Phase 3 neurological investigations are consistent with increased symptoms reporting, minor sensory changes and abnormal EMG results found in other studies of long term health effects associated with OPs. The pattern of the findings is comparable with those seen in neuropathies associated with toxic chemicals such as acrylamide, arsenic and thallium. Distal axonal degeneration is the principal and earliest feature of organophosphate induced delayed neuropathy in both experimental animals [Davis and Richardson, 1980] and humans [Lotti et al., 1984]. In toxic neuropathies, including those related to organophosphate compounds, it is thought that the vulnerability of nerve fibres is related

to axonal length whereby long axons, both motor and sensory, are thought to be more susceptible than shorter axons [Spencer and Schaumburg, 1991]. This assumption has been primarily based on earlier morphological data and more recent electrophysiological, clinical and morphological data suggest that all fibre populations are equally vulnerable [De Rojas and Goldstein, 1990] and that sensory abnormalities are invariably present upon careful clinical examination [Moen, 1991].

8.5 NEUROPSYCHOLOGY

The primary purpose of performing neuropsychological evaluation of the subjects was to test the hypothesis that those individuals who were identified by neurological/neurophysiological examination as 'probable' or 'definite' neuropathy would also show signs of central nervous system damage. The assumption was made that the neuropsychological tests selected would indicate such damage, if it existed.

The first potential limitation is that a selection of tests was used thus reducing the sensitivity of the study. The rationale for their selection has already been discussed. Since the mechanism of action and the type of deficit produced is not known every cognitive function that it is possible to test, should, ideally have been tested. The reasons for not doing so were practical rather than theoretical.

A second potential limitation was sample size. The results show that both age and IQ have significant effects on some aspects of performance, and although these effects can be controlled by statistical method, a more powerful study could have been performed with groups either matched for these variables, or of much larger size. If this had been possible, there would have been less risk that variance due to age and IQ would obliterate differences due to the variables of interest.

Despite these potential limitations, neuropsychological evaluation does indicate a highly significant difference between the three groups. It is clear that the 'Probable/Definite' group reports more psychological symptoms on the self-report scales. The scales are strongly inter-correlated, but two distinct dimensions at least, may be inferred; anxiety and depression. There is also evidence of a graduated effect since the 'Possible' group tend to report more symptoms than the 'No' neuropathy group.

There are three possible theoretical explanations for this phenomenon.

1. Subjects with peripheral nervous system damage also have central nervous system damage, and the latter is manifest as alteration of mood and psychological state. This would have an 'organic" basis that might be conceived of in terms of structural damage and neuronal loss, presumably in sub-cortical or frontal areas since affect is altered, or it might be conceived of in terms of a biochemical effect. This is the most likely explanation.

2. Psychological disturbance may be a secondary effect. Subjects may react with anxiety and low mood to their perceived impairment of peripheral nerve function. Such an effect could be compounded by the study itself; subjects who attended for assessment were aware that they were particularly at risk of such damage, and this itself could produce psychological distress; an inverse placebo effect. This possibility is unlikely given that the subjects were blind to neuropathy category and so there is no reason why the anxiety and depression in the 'Probable/Definite' should have been affected in particular.

3. Subjects were divided into three groups partly as a result of completing a symptom questionnaire, detailing neurophysiological symptoms. Possibly a subset of subjects were more likely to complain in any dimension, and the study has simply used two related methods to identify this group. There is however no evidence to support this.

Although none of these hypotheses can be totally excluded by this study, some strong arguments can be brought to bear against the latter two. Hypothesis 2 assumes that subjects are able to perceive any alteration in peripheral nerve function. Completion of the neurological symptom questionnaire certainly requires this, but the neurophysiological examination relies on tests where the subject is not aware of results and which can detect abnormalities below the threshold of awareness. If subject numbers were not so restricted it would be useful to see if subjects who did not report abnormalities on the neurological questionnaire, but who were symptomatic on the objective tests, were also anxious and depressed.

Hypothesis 3 suggests, in essence, that a sub-group has been identified who are more likely to report symptoms, and that this is quite independent of any effect of organo-phosphates. There is no evidence for this in the present study. If there are individual differences in the amount that people complain, there might also be cultural differences, and so for example the difference between subjects from England and those from Scotland might be affected. Regional differences affecting the neuropsychological data have not been evaluated.

There is some indication that the three groups, 'None' 'Possible' and 'Probable/Definite' are different in terms of their scores on some tasks measuring processing time, but the order effect makes these results hard to interpret. The 'None' group appear to perform worse than the 'Possible' group, while the 'Probable/Definite' group was the slowest. It is unclear whether the slowing of processing speed is another component in a syndrome which includes psychological and neurophysiological symptoms. It may be that a larger sample size would allow better separation of the three groups on these tasks.

Since only one memory task showed a clear difference between the groups, and this was not in the predicted direction, there was no evidence that whatever effect was responsible for the neurophysiological and psychological disturbance also produced memory impairment.

The main conclusion is that amongst the group of subjects identified from Phase 2 of the study, there is a sub-group with evidence of damage to the peripheral nervous system, significant anxiety and depression, and possibly slowing of processing speed but no evidence of memory impairment.

8.6 CONFOUNDERS

The 'no' neuropathy group was on average 8 years younger (mean age 38 years) compared to both the 'possible' and 'probable' neuropathy groups. Age was shown to be associated with increased symptoms reporting and there was an age related increase in sensory thresholds as seen in Phase 2. For neuropsychological investigations, age was always negatively related to memory test variables, suggesting impaired performance with age.

There were found to be no significant differences in IQ across the farming groups participating in Phase 3. Therefore IQ was not an important confounder for the neuropsychological assessments. IQ was most relevant to the neuropsychological investigations, and was found to be positively associated with memory tests and processing speed on univariate analysis.

The Phase 3 neurological symptoms scores confirmed the differences in results between Scotland and England. It was found that the Phase 3 results for English farmers were more reproducible than for Scottish farmers when compared with Phase 2, suggesting that reporting bias alone may not explain the difference between these two regions.

Alcohol consumption was not positively associated with any variable and was not therefore included as a covariate. All of the subjects taking part in Phase 3 ate meat, and so dietary factors alone were not

considered to account for any increase in neuropathy. Individuals with disease or taking medication which might have confounded neuropathy score were excluded from those selected for Phase 3.

Confounders not considered or allowed for in this study are stress which may alter the permeability of the blood-brain barrier, genetic differences, difference in ability to detoxify compounds. These factors might affect the biological dose received by an individual, in addition to that perceived as a measure of 'exposure'.

8.7 KEY FINDINGS

- The neuropathy described in Phase 3 is predominantly of a sensory type both clinically and neurophysiologically and is characteristic of distal, chronic neuropathy with no acute features. Small fibre populations are affected more than large fibre populations. The results of the additional tests (clinical examination and nerve conduction) therefore corroborate the other aspects of the Mayo methods in detecting a possible toxic neuropathy in the clinical studies.

- Increasing severity of neuropathy, as based on symptoms and sensory tests in the clinic was associated with anxiety and depression as measured in the neuropsychological tests. The results did not show that the neuropsychological findings were related to cumulative exposure to OPs, but it was acknowledged that the study design would have limited power to examine such a relationship.

The implications of these findings are considered in more detail in the summary of all three Phases which can be found in this Phase 3 report.

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TABLES

TABLE 5.1 Comparison of field and clinic neuropathy classification

	Clinic			
Field	none	poss	prob/def	all
none	8	9	5	22
poss	5	17	4	26
prob/def	4	12	15	31
all	17	38	24	79

TABLE 5.2 Comparison of field and clinic symptoms score

	Clinic			
Field	0-0.5	1-1.5	≥2	all
0-0.5	35	6	4	45
1-1.5	10	3	4	17
≥2	4	0	13	17
all	49	9	21	79

TABLE 5.3 Comparison of field and clinic heat QST results (0=normal, 1=abnormal)

	Clinic		
Field	0	1	all
0	46	21	67
1	4	8	12
all	50	29	79

TABLE 5.4 Comparison of field and clinic cold QST results (0=normal, 1=abnormal)

	Clinic		
Field	0	1	all
0	20	17	37
1	15	27	42
all	35	44	79

TABLE 5.5 Comparison of field and clinic vibration QST results (0=normal, 1=abnormal)

	Clinic		
Field	0	1	all
0	37	2	39
1	26	14	40
all	63	16	79

TABLE 5.6 Comparison of field and clinic QST thresholds (log scale)

QST	Field			Clinic			Ratio (Field/Clinic)		
	GM	GSD	n	GM	GSD	n	GM	GSD	n
Hot	0.77	3.88	79	1.73	3.10	78	0.44	2.65	78
Cold	0.68	2.76	76	0.54	2.80	75	1.28	2.86	73
Vibration	3.64	3.35	76	1.81	3.48	77	2.06	2.77	75

geometric mean (GM), geometric standard deviation (GSD)

TABLE 6.1 Characteristics of clinical study group

Variable		Clinical classification		
		None	Possible	Prob/definite
Number attending		17	38	24
Number included		17	36	23
Sex	Female	2	5	3
	%	*12*	*14*	*13*
Age	mean	38.8	46.2	46.1
	SD	(13.3)	(10.6)	(11.0)
	min	20	30	22
	max	66	64	62
Alcohol (units/wk)	mean	9.0	10.4	8.4
	SD	(6.7)	(8.5)	(9.8)
	min	0	1	0
	max	20	40	38
Country	Scot	10	28	13
	%	*59*	*78*	*57*
cum. exposure (OPEXP)	mean	1349	4364	1758
	SD	(1638)	(8011)	(1486)
	min	10	68	12
	max	6534	44010	6088

TABLE 6.2 Neurological/Neurophysiological findings

Subject group	*Number in group*	*Neurolog. signs*	*Nerve conduction*	*N. signs or nerve cond.*	*EMG**	*SFEMG*	Sensory abnorm.	Motor abnorm.	**Small fibre abnorm.**	**Large fibre abnorm.**
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%)

*For EMG the *number in group* was 14, 33 and 21 for no, possible and probable/definite neuropathy respectively.

The subject group is according to Phase III classification (based on assessment of neurological symptoms and QST measurements in the clinic)
Cells: Results are expressed as number of subjects with deficit/abnormality and also given as percentage of number in group.
Column headings: (More details can be found in section 6.4.)

Neurolog. signs: Clinical assessment of reflexes, sensation and muscle power.
Nerve conduction: Motor and/or sensory conduction in lower limb
N. signs or nerve cond.: Combination of two previous columns.

This is the most important outcome measure. The subjects included here have neuropathy in a clinical sense and would be reported as such to a referring GP or Physician.

The remaining columns are used to help in characterisation of the neuropathy.

EMG: Needle electromyography of a muscle in the foot (EDB). The results indicate chronic neurogenic changes.
* the *number in group* was 14, 33 and 21 for no, possible and probable/definite neuropathy respectively.
SFEMG: The small numbers indicates that there is only little abnormality in the neuromuscular transmission.
Sensory abnorm: Abnormal sural (sensory) nerve conduction AND on or more abnormal QST thresholds.
Motor abnorm: Abnormal conduction in common peroneal nerve (motor).
Small fibre abnorm: Abnormal hot or cold sensation threshold
Large fibre abnorm: Abnormal vibration sensation threshold or abnormal sural nerve conduction

TABLE 6.3 Estimation of incidence of neuropathy in Phase 2 Farmer population

Subject group	*Number in group*	*Incidence of neuropathy in Phase 3*	*Estimated number with neuropathy in Phase 2*
no neuropathy	125	1/15	8
cold QST abnormal only	247	1/15	16
possible neuropathy	269	10/34	79
probable/ definite neuropathy	44	12/23	23
Total	685	-	**126 (18%)**

Incidence of neuropathy in general population is 0.2% to 0.5%

TABLE 6.4 Symptoms – Phase 3

Subject group	*Number in group*	*Autonomic Symptoms**	*Sensory symptoms*	*Motor symptoms*
no neuropathy	15	0(0 %)	0 (0%)	0(0%)
possible neuropathy	34	1 (3%)	4(12%)	4(12%)
probable/ definite neuropathy	23	6(26%)	18(78%)	11(48%)
Total	72	7(10%)	22(31%)	15(21%)

* Autonomic symptom score of 1 or above.

TABLE 6.5 Subjects with abnormal QST – Phase 3

Subject group	*Number in group*	*Hot threshold*	*Cold threshold*	*Vibration threshold*
no neuropathy	15	0(0)%	0(0%)	0(0%)
possible neuropathy	34	12(35%)	22(65%)	8(24%)
probable/ definite neuropathy	23	14(61%)	17(74%)	5(22%)
Total	72	26(36%)	37(62%)	13(18%)

TABLE 7.1 Neuropsychological test variables. Arrows indicate whether high (↑) or low (↓) values correspond to impaired performance

Test	Variable code	Description
General Health Questionnaire:		
Somatic	GHQ_S	Score 0-7 (↑)
Anxiety and Insomnia	GHQ_AI	"
Social Dysfunction	GHQ_SDY	"
Severe Depression	GHQ_SDP	"
Total	GHQ_TOT	Total of the above scores: 0-28 (↑)
Hospital Anxiety and Depression Scale:		
Anxiety	HAD_A	Score 0-18 (↑)
Depression	HAD_D	Score 0-18 (↑)
Reaction Time:		
Test 1	RT_1	Reaction time (msec) (↑)
Test 2	RT_2	"
Test 3	RT_3	"
Test 4	RT_4	Movement time (msec) (↑)
Test 5	RT_5	"
Match-to-Sample Visual Search:	VS_TC	Number correct out of 52 trials (↓)
total correct	VS_DT	Mean decision time (msec) (↑)
decision time	VS_MT	Mean movement time (msec) (↑)
movement time		
Pattern Recognition:		
total correct	PR_TC	Number correct out of 20 trials (↓)
mean reaction time	PR_RT	Mean reaction time (msec) (↑)
Spatial Recognition:		
total correct	SR_TC	Number correct out of 20 trials (↓)
mean reaction time	SR_RT	Mean reaction time (msec) (↑)
Paired Associte Learning:		
total correct	PAL_TC	Number correct out of 32 trials (↓)
Spatial Span	SPAN	Length of longest sequence recalled (↓)
Rey Auditory Verbal Learning	RAVL	Number correct out of 75 (↓)

TABLE 7.2 Psychological symptom scale variables. Standard errors in parenthesis.

Variable	F-test	Adjusted group means			Group mean order
	P-value	None (1)	Poss (2)	Prob (3)	(high to low)
GHQ_S	0.000	0.090 (0.42)	0.65 (0.28)	2.77 (0.36)	3 > 2 = 1
GHQ_AI	0.009	0.68 (0.48)	1.09 (0.33)	2.51 (0.41)	3 > 2 = 1
GHQ_SDY	0.029	0.46 (0.38)	0.66 (0.25)	1.64 (0.32)	3 > 2 = 1
GHQ_SDP	0.136	0.04 (0.23)	0.22 (0.15)	0.62 (0.20)	3 = 2 = 1
GHQ_TOT	0.000	2.07 (1.13)	2.63 (0.77)	7.59 (0.97)	3 > 2 = 1
HAD_A	0.001	4.59 (0.81)	5.97 (0.55)	8.68 (0.70)	3 > 2 = 1
HAD_D	0.000	3.22 (0.70)	3.22 (0.47)	6.97 (0.60)	3 > 2 = 1

> significantly greater than
= not siginficantly different to

TABLE 7.3 Partial correlations among psychological symptom scale variables.

GHQ_S	1.00						
GHQ_A	0.44	1.00					
GHQ_SDY	0.35	0.56	1.00				
GHQ_SDP	0.21	0.30	0.49	1.00			
GHQ_TOT	0.71	0.84	0.80	0.58	1.00		
HAD_A	0.26	0.52	0.37	0.26	0.49	1.00	
HAD_D	0.32	0.41	0.40	0.48	0.53	0.46	1.00
	GHQ_S	GHQ_A	GHQ_SDY	GHQ_SDP	GHQ_TOT	HAD_A	HAD_D

TABLE 7.4 Processing time variables (log transformed). Standard errors in parenthesis.

Test	F-test	Adjusted group means			Group order
	P-value	None (1)	Poss (2)	Prob (3)	(high to low)
RT_1	0.358	6.722 (0.050)	6.644 (0.034)	6.703 (0.043)	1 = 3 = 2
RT_2	0.052	6.724 (0.038)	6.667 (0.025)	6.767 (0.032)	3 = 1 = 2 (3 > 2)
RT_3	0.019	5.829 (0.054)	5.764 (0.037)	5.934 (0.046)	3 = 1 = 2 (3 > 2)
RT_4	0.101	6.321 (0.045)	6.236 (0.030)	6.331 (0.039)	3 = 1 = 2
RT_5	0.071	6.282 (0.050)	6.181 (0.034)	6.295 (0.043)	3 = 1 = 2
VS_DT	0.090	7.634 (0.065)	7.651 (0.044)	7.794 (0.056)	3 = 2 = 1
VS_MT	0.435	6.526 (0.049)	6.452 (0.033)	6.494 (0.042)	1 = 3 = 2
PR_RT	0.045	7.885 (0.060)	7.747 (0.040)	7.891 (0.051)	3 = 1 = 2
SR_RT	0.007	7.702 (0.061)	7.516 (0.041)	7.701 (0.052)	1 = 3 > 2

> significantly greater than
= not siginficantly different to

TABLE 7.5 Partial correlations among processing time variables.

RT_1	1.00								
RT_2	0.62	1.00							
RT_3	0.35	0.48	1.00						
RT_4	0.43	0.70	0.50	1.00					
RT_5	0.47	0.73	0.43	0.84	1.00				
VS_DT	0.23	0.27	0.20	0.32	0.38	1.00			
VS_MT	0.29	0.50	0.24	0.50	0.54	0.20	1.00		
PR_RT	0.34	0.47	0.24	0.50	0.47	0.37	0.36	1.00	
SR_RT	0.35	0.37	-0.01	0.20	0.33	0.34	0.30	0.61	1.00
	RT_1	RT_2	RT_3	RT_4	RT_5	VS_DT	VS_MT	PR_RT	SR_RT

TABLE 7.6 Memory variables. Standard errors in parenthesis.

a) Binomial responses

Test	χ^2-test	Adjusted group means			Group order
	P-value	None (1)	Poss (2)	Prob (3)	(high to low)
VS_TC	0.281	0.94 (0.01)	0.94 (0.01)	0.96 (0.01)	3 = 1 = 2
PR_TC	0.848	0.52 (0.03)	0.51 (0.02)	0.53 (0.02)	3 = 1 = 2
SR_TC	0.956	0.52 (0.03)	0.53 (0.02)	0.53 (0.02)	2 = 3 = 1
PAL_TC	0.035	0.62 (0.02)	0.66 (0.01)	0.69 (0.02)	3 = 2 = 1 (3 > 1)

b) Normal responses

Test	F-test	Adjusted group means			Group order
	P-value	None (1)	Poss (2)	Prob (3)	(high to low)
SPAN	0.789	5.80 (0.32)	6.07 (0.21)	5.96 (0.27)	2 = 3 = 1
RAVL	0.411	50.8 (2.2)	49.5 (1.5)	47.1 (1.9)	1 = 2 = 3

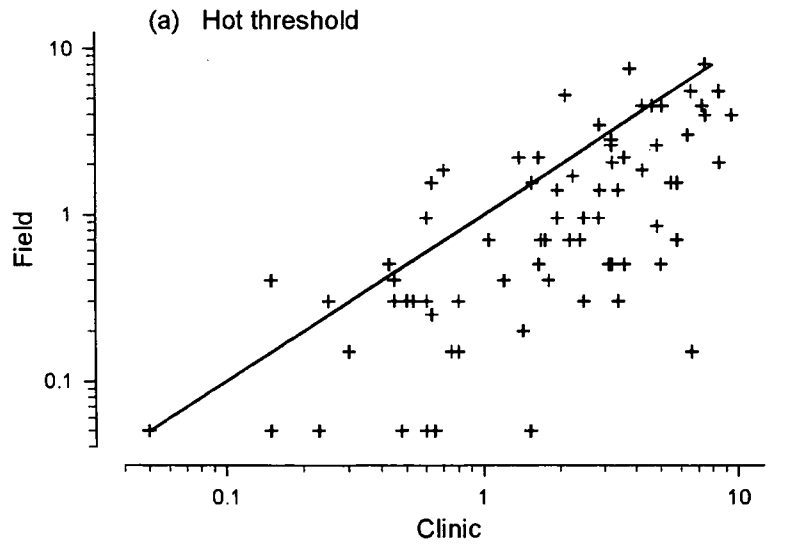
> significantly greater than
= not siginficantly different to

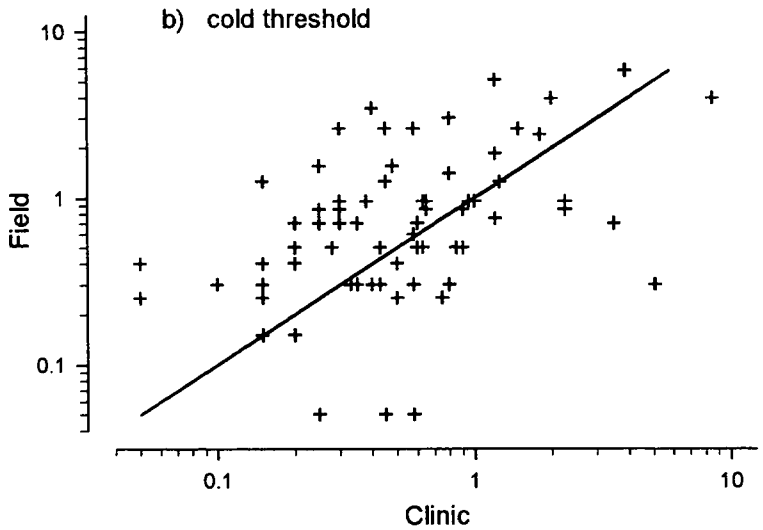
TABLE 7.7 Partial correlations among memory variables.

VS_TC*	1.00					
PR_TC*	-0.15	1.00				
SR_TC*	0.14	0.31	1.00			
PAL_TC*	-0.05	0.36	0.28	1.00		
SPAN	0.28	0.07	0.10	0.22	1.00	
RAVL	-0.12	0.15	0.04	0.47	0.10	1.00
	VS_TC*	PR_TC*	SR_TC*	PAL_TC*	SPAN	RAVL

* deviance residuals

FIGURES





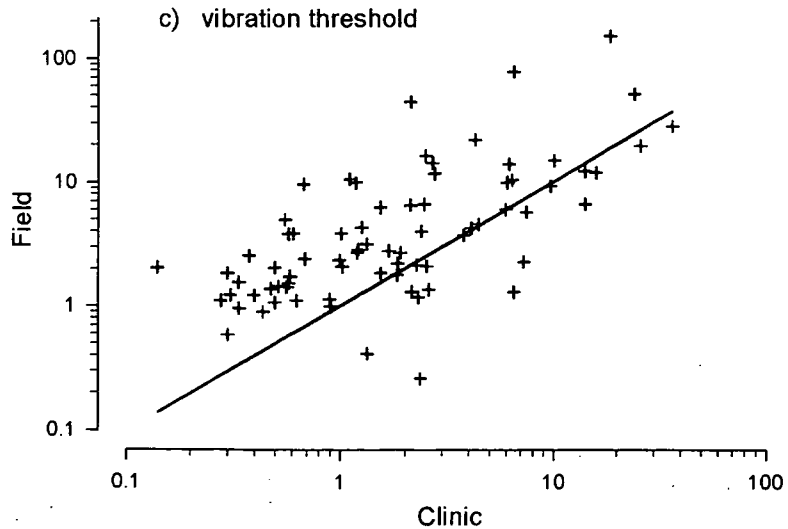


Figure 5.1 Comparison between field (phase 2) and clinic (phase 3) measurements of sensory thresholds

Figure 6.1 Age distributions within clinical neuropathy categories

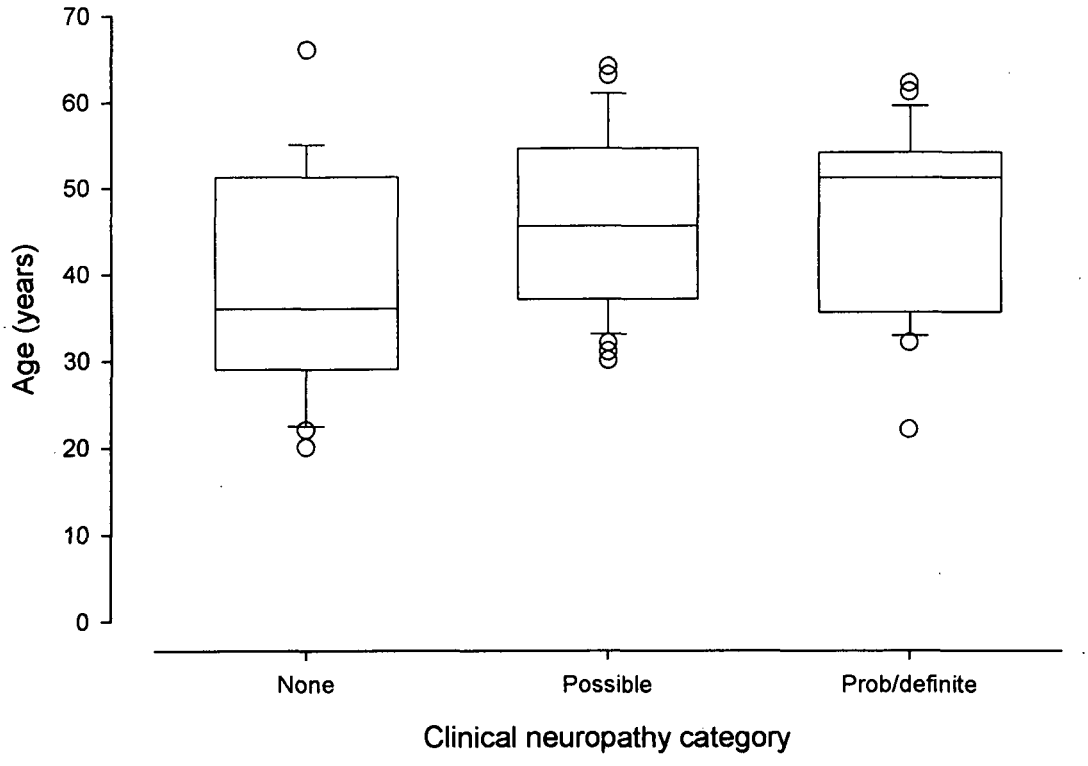
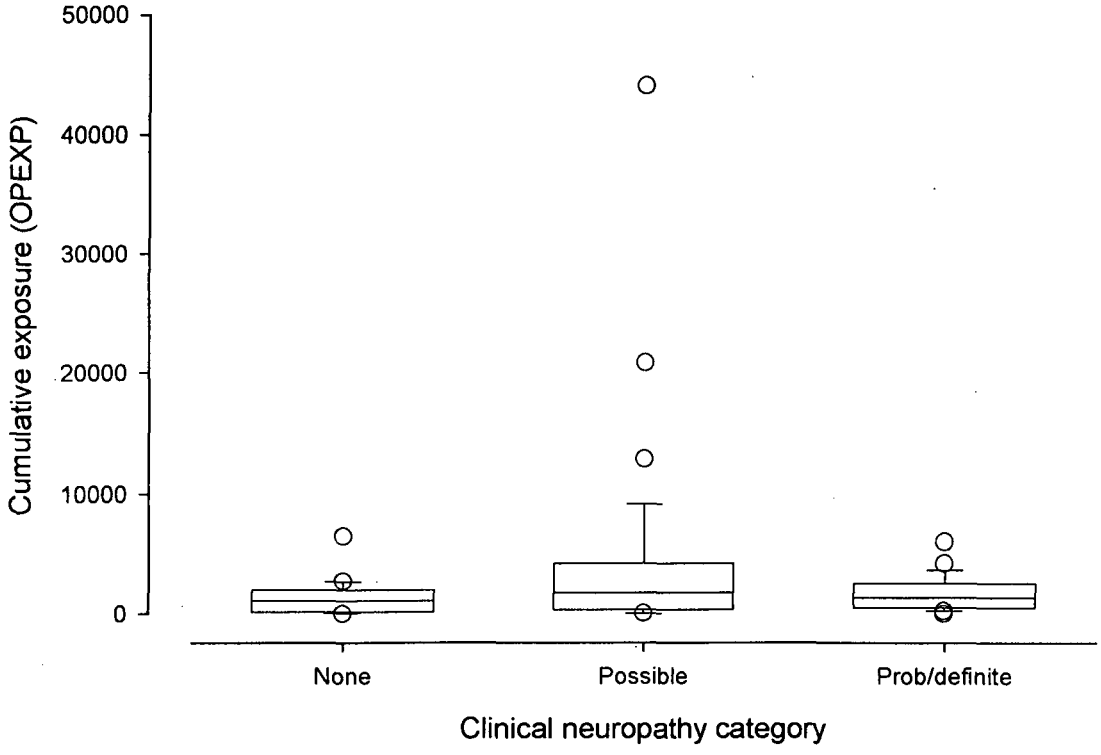


Figure 6.2 Cumulative exopsure distributions within clinical neuropathy categories



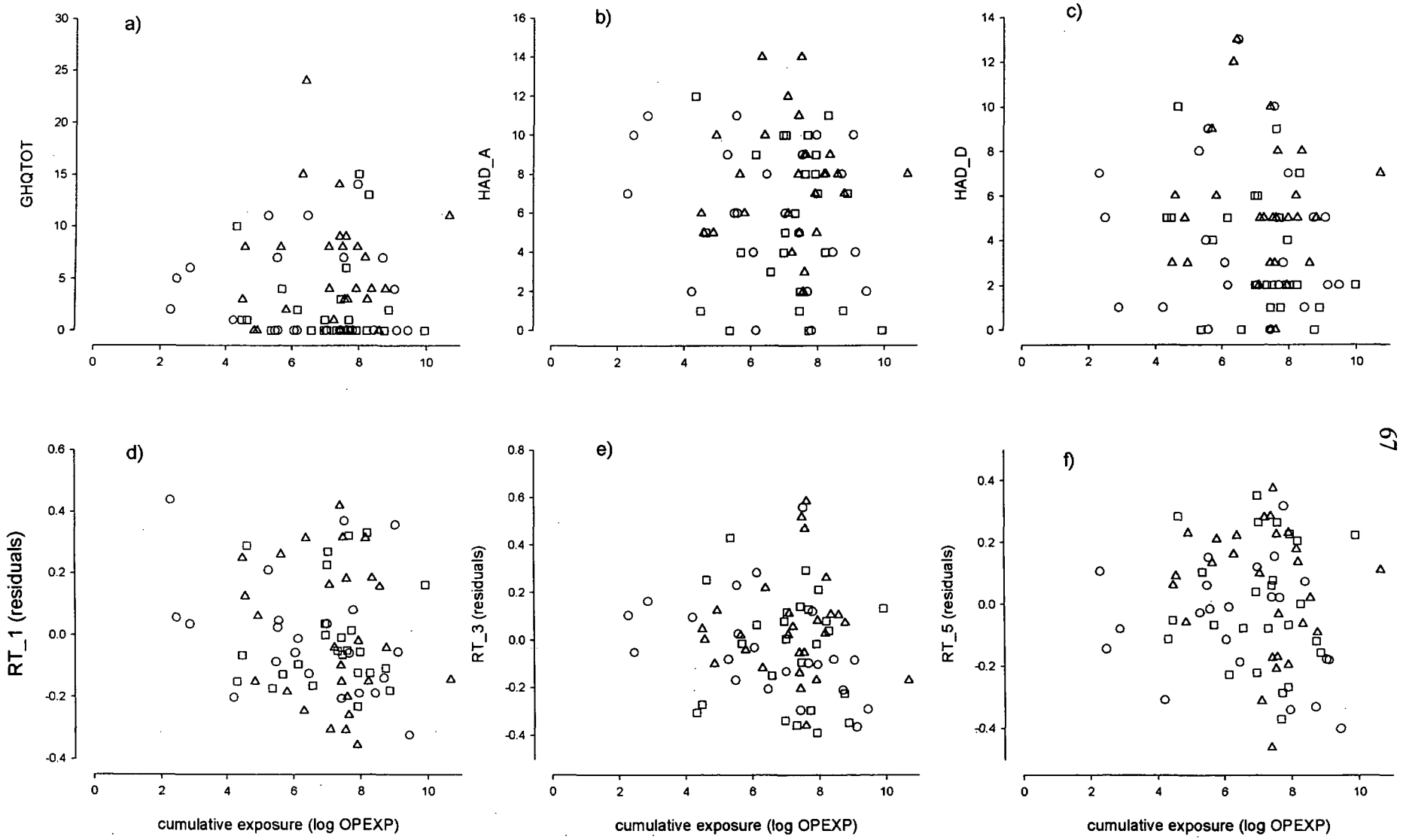


Figure 7.1 Relationships between neuropsychological variables and cumulative exposure. Variables marked '(residuals)' have been adjusted for age and IQ. Symbols denote neuropathy category used for selection to phase 3: (○) none, (□) possible, (△) probable/definite

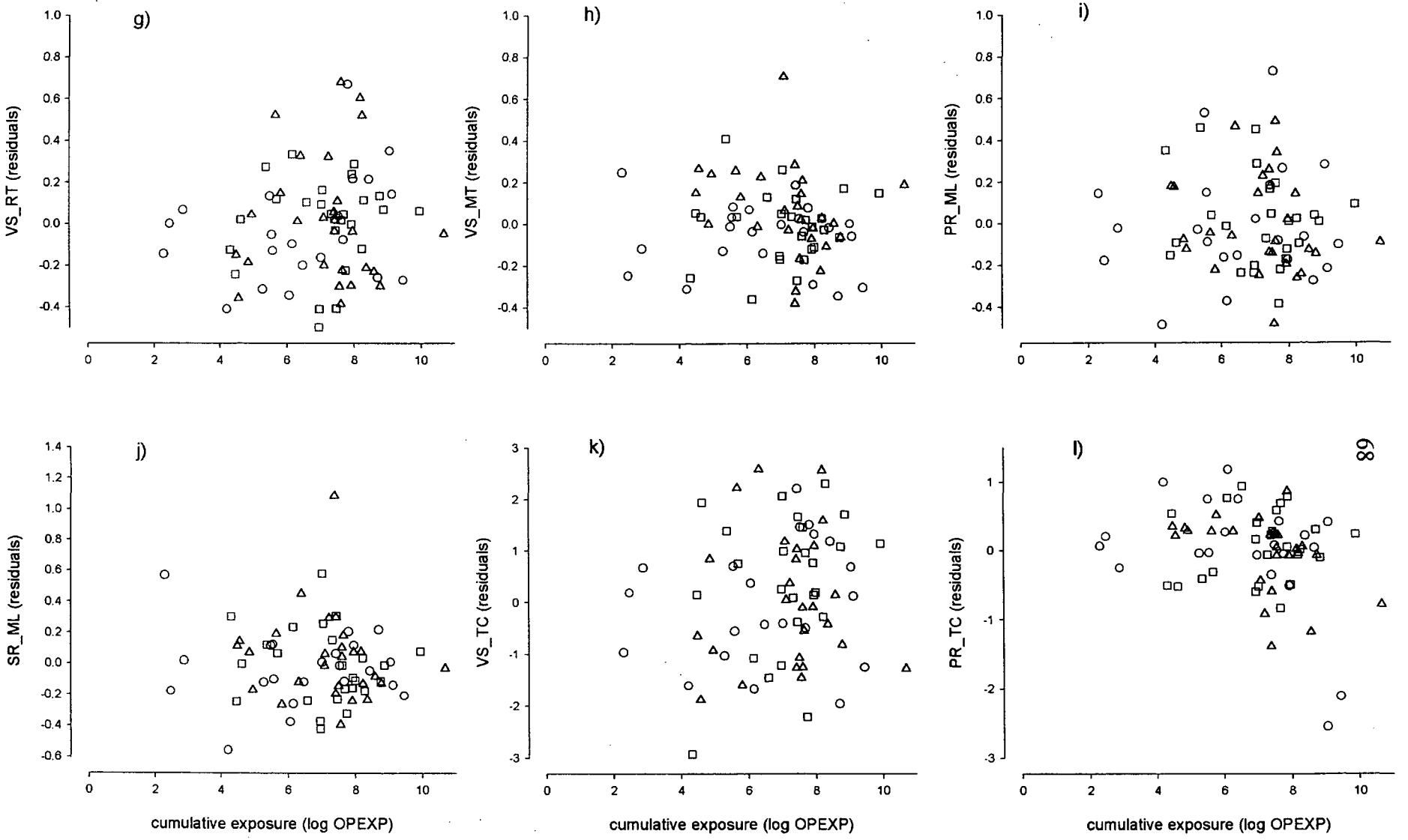


Figure 7.1 (continued)

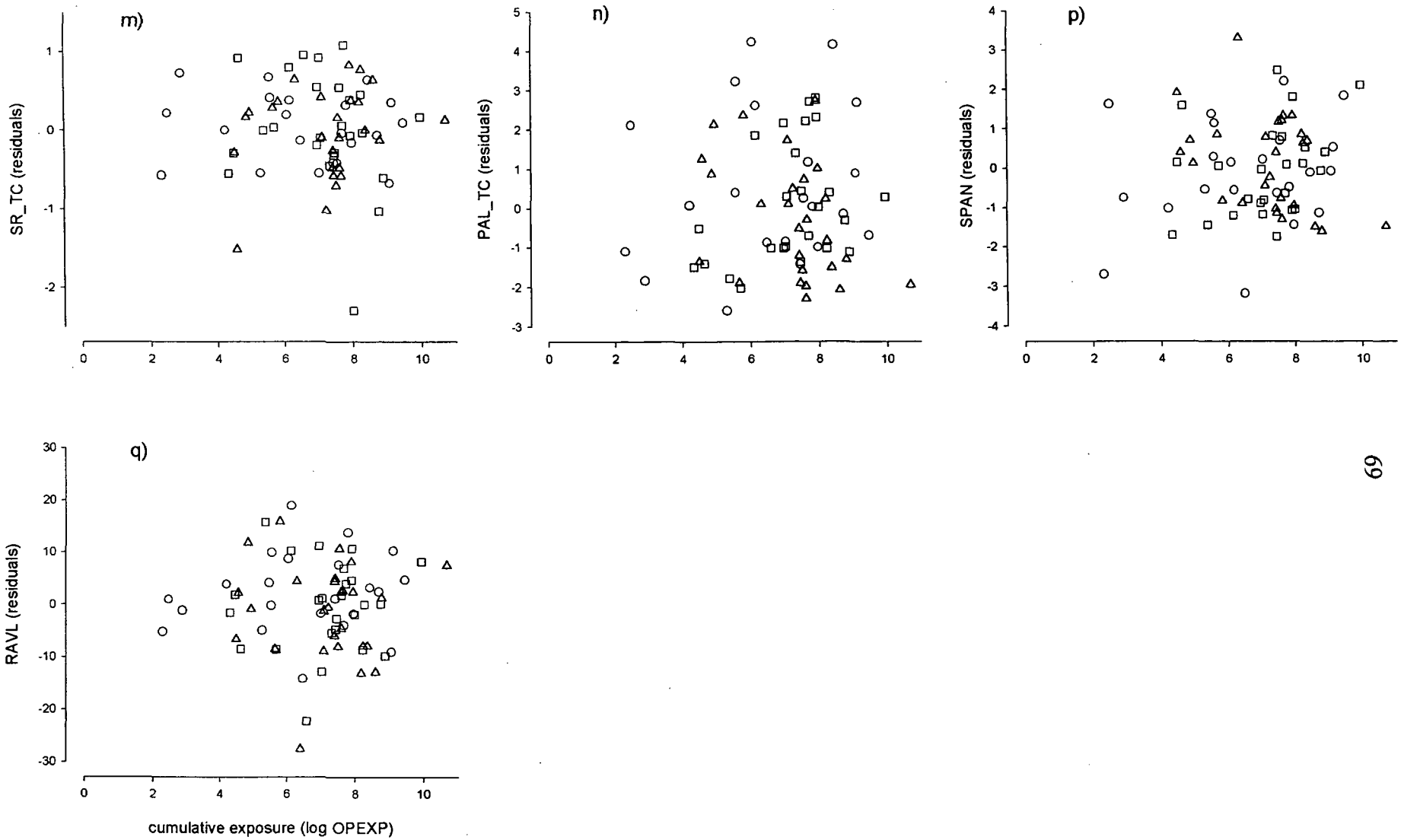


Figure 7.1 (continued)

APPENDICES

APPENDIX 1

Neurological Questionnaire and Protocol

Protocol for the completion of the neuropathy symptoms questionnaire and for performing the thermal and vibration sensory tests.

GENERAL INSTRUCTIONS

The subject should be informed that the majority of questions require a simple "yes" or "no" answer. The actual printed words should be used for each question. The questionnaire is divided into sections, each with a preamble to introduce the subject to the kind of questions that will be asked.

Questions about a specific symptom at a specific site consist of a main question followed by explanations or one or more subsidiary questions giving a different manifestation of the same symptom. If the answer to all of these questions is "no" go on to the next site/symptom. If the answer to any of these questions is "yes", further questions are asked to exclude :

1) temporary disabilities from which most people suffer some time or other during their life without having neuropathy (e.g. pin and needles in the hand after lying on the arm) or,
2) asymmetrical symptoms which are unlikely in a toxic neuropathy, of the type which may be associated with exposure to organo-phosphates.

RECORDING THE REPLIES TO THE QUESTIONS

The questionnaire has been set out to facilitate transfer of data to computer. Most questions are of the yes/no type and replies to these question should be recorded as ticks in the appropriate box. Where the answer to the question is a number, e.g. How long have you had this weakness? [in months] the number should be recorded directly in the boxes provided. Where the question are of a more open type e.g. name of medication, the reply should be recorded in full and coding will be assigned later.

The results of the sensory test should be entered on the special *Sensory Test Record Sheet.*

General information

Date of assesment Each box should be filled e.g. 1st November 1997 should be recorded as 011197.

Subject's name and code number insert the subject's name and corresponding code number. Where father and son have the same name, record senior and junior as appropriate.

Date of birth Each box should be filled e.g. 7 August 1952 should be recorded as 070852.

Investigator Insert initials of person performing the assessment in the space provided.

COMMENTS ON SECTIONS OR INDIVIDUAL QUESTIONS

N.B. For all relevant sections, if the reply to "How long have you had this .....?" is less than one full month, record a zero.
The notes in the 'general instructions' section should be referred to when completing the 'scored symptoms' section of the questionnaire.

Muscle weakness (sections 1, 2, 3 & 4)

The preamble is used to make sure that the subject understands what is meant by weakness.

The questions are designed to consider weakness occurring in both upper and lower limbs, to

establish how long the weakness has been present and to distinguish weakness associated with neuropathy caused by painful conditions, such as arthritis.

The two or three additional questions following question (a) in each section give examples of the type of problem which might be noticed due to weakness in that specific part of the body, and help the subject to understand the information which is being sought.

Section 3a; muscle weakness in toes or feet Examples of this problem are given in questions 3b and c. In question 3c 'walking on heels' may be something that the subject has not tried before, and it is reasonable for them to test this ability at the time of the assessment.

Negative sensory symptoms (sections 5 & 6)

This covers loss of sensation and the preamble explains the different terms that may be used to describe these symptoms.

Section 5b; difficulty feeling objects This includes being able to tell for example whether an object has sharp edges or is cold. It should be distinguished from being able to recognise what an object is by its size and shape, without looking at the object. (This ability is covered later in the questionnaire).

Section 5i; do any of the problems extend up the forearm
This question aims to identify whether subjects with 'yes' answers to questions 5a to 5c, experience similar symptoms of numbness in the forearm.

Section 6b: ability to feel your feet or the ground when walking This question seeks the same type of information as 5b. Again subjects may consciously have to think about whether they are aware of their feet being in contact with the ground when walking. People who lack this ability often describe themselves as 'walking on cotton wool'.

Positive sensory symptoms (sections 7 & 8)
This section is used to record the presence of unusual sensations.

Section 7a-c: Only record a "yes" if the subject has the specific symptom mentioned in the question. Further explanation should not be offered at this stage. Other similar symptoms may be covered in Section 7h.

Section 7d:spontaneous pain in the hands This refers to pain which is not associated with any activity, and can vary in nature from sharp to dull or burning.

Section 7e: asks about painful sensations occurring either spontaneously or when the subject performs tasks with his/her hands that should not normally be painful, for example picking up a coin or a pillow.

Section 7f and 7g: are used to characterise further a painful sensation reported by the subject in 7e.

Section 7h: Read the list of sensations to the subject, and circle only those symptoms to which the subject gives a "yes" answer. **Further explanation should not be offered for this question.**

Section 8: As section 7, but for feet.

Section 8e: An example might be touching bedclothes or a soft carpet.

Autonomic (sections 9, 10, 11, 12 & 13)

This covers the part of the nerve system that control "automatic" functions of the body over which we exert little or no voluntary control. The preamble prepares the subject for some questions which may be embarrassing. Stress the importance of obtaining answers to all questions. If the subject declines to answer, offer him/her that page of the questionnaire for self completion there and then. When page is returned insert it into the folder without scrutinising the replies. Later, the relevant sections should be marked subject-completed or reply declined as appropriate.

Section 9: Postural hypotension
This is a fall in blood pressure associated with a change in position , for example, from lying to standing or standing to sitting. Individuals may experience lightheadedness, or may faint.

If the subject answers "yes" to 9a or 9b, the subsequent questions explore how often these symptoms occur, and how long they have been present. You may offer as examples the changes in position listed.

Section 10: Night diarrhoea This is the passage of a loose or watery motion during the night, at least once a week. Any pattern associated with dietary intake or alcohol should be excluded.

Section 11a: Loss of urinary control
Ask the alternative question "Do you have problem with your water works" if the subject has difficulty understanding the information which is being sought.

Section 11c: The purpose of this question is to exclude stress incontinence which may occur in women after child birth.

Section 11e to k These questions consider specific problems associated with autonomic bladder dysfunction, and seek to exclude other conditions resulting in loss of urinary control.
Possible additional explanations are as follows:
11e. Being aware of the sensation of passing urine , not just the sound
11f. Can you feel the toilet paper moving over your skin
11g. Do you sometimes wet yourself and not know that it has happened
11h. After passing urine do you feel comfortable, as though your bladder is empty
11i. Do you have any difficulty getting started
11k. Can you wait a while or do you need to pass urine quickly when you feel you want to go.

Section 12: Sexual function The questions in this section should be handled discreetly.
Self completion by the subject is acceptable as outlined above.
Section 12a and 12b are applicable to men only.

Section 13: Sweating
These questions ask about unusual patterns of sweating, either too little or too much both in hands and feet.

Other information

Breathlessness
Section 14: The pace at which the subjects normally walks is their own walking pace.

Smoking and alcohol consumption
Section 15 and 16: Enter the average values for cigarette and alcohol consumption on a daily and weekly basis respectively.

Medication

Section 17: The subject should have had prior warning that information about medication will be asked. Please record details given by subject or from medicine containers the subject might bring along. If any detail is not known, leave the corresponding section blank.

Family history

Section 18: Record "don't know "responses as "no". If necessary repeat the explanations: muscle weakness means loss of power or strength right from the beginning of attempting to do something. Sensory loss refers to numbness, loss of feeling or deadness.

Section 19: This question seeks information about a specific neurological condition which may be associated with neuropathy. If the answer is "yes" to main question read the categories of relatives to the subject.

Relevant medical history

Section 20: Multiple Sclerosis (MS) and epilepsy are examples of neurological diseases.

Section21:

Ask whether the condition was diagnosed at a hospital or by the GP (family doctor).Place a tick in the appropriate box

Section 22: Enquire specifically about diabetes and thyroid disease as these may be associated with neuropathy. Make a note of other diseases for which the subject is receiving treatment.

Neuropathy Symptoms Questionnaire

Preliminary Information

0.1 Site number. | | |

0.2 Date of survey (ddm myy) | | | |

0.3 Subject's Name and Code

Surname	Forenames	Code

0.4 Other Information

Date of Birth | | | Gender M / F |

Height m | • | | Weight kg | | |

Are you right or left handed ? (*which hand do you write with)* R/L

Do you eat meat, poultry or fish? Y/N

Use of vibrating equipment

0.5 Does your work regularly involve the use of the following equipment?

	Hours/day	Years in job
Chain saws/ wood work machines		
Power hammers/percussive drills		
Grinding and rotary tools		
Riveting tools		
Fork lift driving		
Tractor/ quad bike driving		

Hobbies:

0.6 Do you take part in any of the following at least once per week?

	Hours/week
Motorcycling or Mountain biking	
Wood working	
Vehicle maintenance	

Highest level of education

0.7 What was the highest qualification you obtained at school or college? (*tick one box*)

No certificates	
O-level/standard grade	
A-level/Highers	
College/University	

Scored symptoms

Muscle weakness

I am going to ask you questions about any weakness in your muscles. Weakness means loss of power or strength right from the beginning of attempting to do something. It should not be confused with fatigue or tiredness. (*Fatigue is used to describe inability to sustain muscle activity which initially was of normal strength*).

Hands:		Yes	No
1a	**Do you have a muscle weakness in the hands?** (*Have you noticed any difficulty with the strength of your hand grip?*)	☐	☐
1b	Do you have difficulty unscrewing tops of jars due to finger or hand weakness?	☐	☐
1c	Do you have difficulty buttoning or unbuttoning shirts/clothes?	☐	☐
	If yes to any above:		
1d	Do you have difficulty with weakness in both hands?	☐	☐
1e	Have the problems been there for the last month?	☐	☐
1f	How long have you had this weakness? [in months]	_____	
1g	Do you have pain in the hands?	☐	☐
	If yes,		
1h	Do you think your weakness is caused by the pain alone?	☐	☐

Shoulders:		Yes	No
2a	**Do you have a muscle weakness in the upper arms or shoulders?**	☐	☐
2b	Do you have difficulty lifting your arms to reach objects on high shelves?	☐	☐
2c	Do you have difficulty brushing your hair?	☐	☐
2d	Do you have difficulty putting on your jacket?	☐	☐
	If yes to any above:		
2e	Do you have difficulty with weakness on both sides?	☐	☐
2f	Have the problems been there for the last month?	☐	☐
2g	How long have you had this weakness? [in months]	_____	
2h	Do you have pain in the arms or shoulders?	☐	☐
	If yes,		
2i	Do you think your weakness is caused by the pain alone?	☐	☐

	Feet:	Yes	No
3a	**Do you have muscle weakness in the toes or feet?**	☐	☐
3b	Are you unable to walk on tiptoes ?	☐	☐
3c	Are you unable to walk on your heels ?	☐	☐
	If yes to any above:		
3d	Do you have difficulty with weakness in both feet?	☐	☐
3e	Have the problems been there for the last month?	☐	☐
3f	How long have you had this weakness? [in months]	_____	
3g	Do you have pain in the feet?	☐	☐
	If yes,		
3h	Do you think your weakness is caused by the pain alone?	☐	☐

	Legs/hips:	Yes	No
4a	**Do you have a muscle weakness in the legs or hips?**	☐	☐
4b	Do you have difficulty in climbing stairs?	☐	☐
4c	Do you have difficulty in rising from a low chair?	☐	☐
4d	Do you have difficulty in getting into or out of a bath without help because of muscle weakness (not because of loss of balance or pain) ?	☐	☐
	If yes to any above:		
4e	Do you have difficulty with weakness on both sides?	☐	☐
4f	Have the problems been there for the last month?	☐	☐
4g	How long have you had this weakness? [in months]	_____	
4h	Do you have pain in the legs or hips?	☐	☐
	If yes,		
4i	Do you think your weakness is caused by the pain alone?	☐	☐

Negative sensory symptoms

I am going to ask you about numbness which you might call loss of feeling, insensitivity or deadness. I will start with the hands.

	Hands:	Yes	No
5a	**Do you have numbness of the hands?**	☐	☐
5b	**Do have you difficulty feeling objects with your hands?**	☐	☐
5c	**Are you unable to distinguish hot from cold water with your hands?**	☐	☐
	If yes to any above:		
5d	Do any of these occur only occasionally and only last a few minutes?	☐	☐
5e	Do you have this problem in both hands?	☐	☐
5f	Have the problems been there for the last month?	☐	☐
5g	How long have you had this abnormal sensation? [in months]	_____	
5h	Are any of the problems associated with discomfort or pain?	☐	☐
5i	Do any of the problems extend up the forearm?	☐	☐

I am now going to ask you about numbness or what you might call loss of feeling, insensitivity or deadness affecting your feet.

	Feet: Yes	No
6a	**Do you have numbness of the feet?**	☐ ☐
6b	**Are you unable to feel your feet or the ground when walking?**	☐ ☐
6c	**Are you unable to distinguish hot from cold when taking a bath?**	☐ ☐
	If yes to any above:	
6d	Do any of these occur only occasionally and only last a few minutes ?	☐ ☐
6e	Do you have this problem in both feet?	☐ ☐
6f	Have the problems been there for the last month?	☐ ☐
6g	How long have you had this abnormal sensation? [in months]	_____
6h	Are any of the problems associated with discomfort or pain?	☐ ☐
6i	Do any of the problems extend up the leg?	☐ ☐

Positive sensory symptoms

I am going to ask you about any pain or other peculiar or unusual sensations you might have. I will start with the hands.

	Hands:	Yes	No
7a	**Do you have "burning discomfort" in the hands?**	☐	☐
7b	**Do you have prickling sensation in the hands?**	☐	☐
7c	**Do you have pins and needles or tingling in the hands?**	☐	☐
7d	**Do you have spontaneous pain in the hands (jabbing, stabbing, burning, dull, sharp,toothache-like)?**	☐	☐
7e	**Do you have painful unpleasant sensations in the hands for example when touching nonpainful things?**	☐	☐
7f	*if yes*, is the pain continuous rather than occurring intermittently?	☐	☐
7g	*if intermittent*, how long do the attacks last? [in hours]	_____	

7h **I am going to read out a list of types of pain or other strange sensations you may have in your hands: Please say yes to any that apply to you.** ☐ ☐

(Operator: Please circle the symptoms present)

jabbing or stabbing pain	tingling/prickling	aching or hurting
burning/excessively warm	excessively cold	tight/tightly wrapped
too sensitive	pain	

If yes to any of the above (a-h) :

7i	Do any of these problems occur only occasionally and only last a few minutes ?	☐ ☐
7j	Do you have the problems in both hands?	☐ ☐
7k	Have the problems been there for the last month?	☐ ☐
7l	How long have you had this type of discomfort [in months]?	_____

	Feet:	Yes	No
8a	**Do you have "burning discomfort" in the feet?**	☐	☐
8b	**Do you have prickling sensation in the feet?**	☐	☐
8c	**Do you have pins and needles or tingling in the feet?**	☐	☐
8d	**Do you have spontaneous pain in the feet (jabbing, stabbing, burning, dull, sharp,toothache-like)?**	☐	☐
8e	**Do you have painful unpleasant sensations in the feet for example when touching nonpainful things?**	☐	☐

8f *if yes*, is the pain continuous rather than occuring in attacks? ☐ ☐
8g *if attacks*, how long do they last? [in hours] _____

8h **I am going to read out a list of types of pain or other strange sensations you may have in your feet: Please say yes to any that apply to you.** ☐ ☐
(Operator: Please circle the symptoms present)

jabbing or stabbing pain	tingling/prickling	aching or hurting
burning/excessively warm	excessively cold	tight/tightly wrapped
too sensitive	pain	

If yes to any of the above (8a-h) :

8i Do any of these problems occur only occasionally and only last a few minutes at a time? ☐ ☐
8j Do you have the problems in both legs? ☐ ☐
8k Have the problems been there for the last month? ☐ ☐
8l How long have you had this type of discomfort [in months]? _____

Autonomic

I am going to ask you a number of questions; some may be difficult or embarassing to answer. They include questions about bowel, bladder and sexual function. They are important and I would be grateful if you can answer them. If you feel some are too private then you can say that you prefer not to answer.

Postural hypotension/fainting:
First some questions about fainting or light headedness.

		Yes	No
9a	**Do you feel light headed when you suddenly change your position** (from lying to sitting position or from lying/sitting position to standing)	☐	☐
9b	**Do you faint when you suddenly change your position** (from lying to sitting position or from lying/sitting position to standing)	☐	☐
	If yes to any of the above:		
9c	Has the problem been there for the last month?	☐	☐
9d	Have you fainted/felt light headed more than once during the last year?	☐	☐
9e	Often while standing?	☐	☐
9f	Often while seated?	☐	☐
9g	Often while lying?	☐	☐
9h	How often [number of times per week]?	_____	
9i	How long have you had these attacks of fainting/light headedness [in months]	_____	

Night diarrhoea

Now some questions about diarrhoea (passing watery or loose stool).

		Yes	No
10a	**Do you have night diarrhoea?**	☐	☐
	If yes to the above:		
10b	Has the problem been there for the last month?	☐	☐
10c	Does this occur frequently (every night)?	☐	☐
10d	How many nights during the week?	____	
10e	How long have you had this problem? [in months]	____	

Loss of urinary control

Now some questions about water works/bladder function.

		Yes	No
11a	**Do you have loss of control of bladder function?** (Alternative question: Do you have a problem with your water works?)	☐	☐
	If yes to the above:		
11b	Has the problem been there for the last month?	☐	☐
11c	*(For females)* Does the problem only occur when you cough, sneeze, strain or push?	☐	☐
11d	How long have you had this problem [in months]	____	
11e	Can you feel yourself passing urine?	☐	☐
11f	Can you feel when wiping yourself?	☐	☐
11g	Do you leak without knowing it?	☐	☐
11h	Do you feel your bladder is empty after you have passed urine?	☐	☐
11i	Do you have difficulty starting to pass urine?	☐	☐
11j	Do you wet the bed at night?	☐	☐
11k	Can you pass or hold urine when you want to ?	☐	☐

Impotence

Now some questions about sexual function. If you find these too embarassing, you can fill in the answers yourself on the questionnaire.

		Yes	No
12a	**(*For males*) Are you unable to have an erection of the penis?**	☐	☐
12b	**(*For males*) Are you unable to ejaculate or come (emission of fluid with sexual climax)?**	☐	☐
12c	**Do you have loss of sexual desire?**	☐	☐
	If yes to any of the above:		
12d	Has the problem been there for the last month?	☐	☐
12e	How long have you had this problem [in months]	____	

Sweating

Now some question about sweating.

	Yes	No
13a Have you noticed lack of sweating in your hands?	☐	☐
13b Have you noticed lack of sweating in your feet?	☐	☐
13c Do you feel you overheat because you sweat insufficiently?	☐	☐
13d Do you sweat too much in your hands?	☐	☐
13e Do you sweat too much in your feet?	☐	☐
If yes to any of the above:		
13f Do you have this problem on both sides	☐	☐
13g Has the problem been there for the last month?	☐	☐
13h How long have you had this problem [in months]	____	

Other information

	Yes	No
14. Do you have to stop for breath when walking at your own pace on level ground ?	☐	☐
15a. Do you smoke?	☐	☐
If yes,		
How many manufactured cigarettes/cigars do you smoke each day?	____	
How many ounces of tobbaco do you smoke each week? *Pipe/ roll-ups*	____	
If no,		
15b. **Have you ever smoked?**	☐	☐
If yes, in what year did you stop	____	
16. Do you drink alcohol?	☐	☐
If yes, number of pints beer or lager /week	____	
number of glasses wine or spirits / week	____	

	Yes	No
17. Are you on medication?	☐	☐
If yes, please complete below		

name of medication	*size of tablet (mg)*	*number of tablets/day*	*since which date*
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

	Yes	No
18. Does anyone in your family have muscle weakness or sensory loss?	☐	☐
19. Do any of your relatives have excessively high arches of the feet with or without curled toes?	☐	☐

If yes, which of the following are affected? (Operator: please circle)

grandparents, uncles or aunts, parents, brothers or sisters, children, nephews or nieces, grandchildren

	Yes	No
20. Has a Doctor ever told you that you have a neurological disease?	☐	☐

If yes, please complete:
Name of disease: _____
Duration: _____

	Hospital	GP
21. Who diagnosed the condition?	☐	☐

	Yes	No
22. Do you have any other disease?	☐	☐

If yes, please complete:

	Type	Duration
Diabetes	_____	_____
Thyroid	_____	_____
Other	_____	_____

Protocol for recording signs of neuropathy

Neuropathy signs will be recorded by medically qualified staff (Dr Aziz or Dr Julu) using standard medical procedures (Mayo Clinic). Where no side is indicated, the right side will be tested unless the neuropathy questionnaire indicates that this side is affected asymmetrically. In this case, the left side will be used.

Cranial Nerves

Cranial nerves III, IV, VI, V, VII, X and XII are tested bilaterally using standard methods (Mayo Clinic).

Reflexes

Reflexes are tested for each muscle by applying a small tap with a tendon hammer to its tendon. The muscles selected allows testing of reflexes in upper and lower limb, proximal and distal muscles.

Score	*Reflex*
0	normal response
1	response present only on reinforcement by contraction of other muscle groups
2	no response, even with reinforcement

Sensation

Type	*Method of testing*
Pin prick	Special disposable sharp pin
Vibration	Tuning fork (frequency 128 Hz)
Fine touch	Cotton ball or camel hair brush
Position	Standard procedures (Mayo Clinic)

The scoring of the clinical tests of sensation reflects the distal distribution of an axonal neuropathy. Normally, the problem develop first distally in the lower limb, thereafter the deficit move up the lower limb. Next the upper limb is affected and the scoring system reflects the phenomenon.

Muscle Power

Muscle power is assessed by asking the subject to move the particular muscle or muscle group against resistence applied by the investigator. The assessment is performed in the distal, intermediate and proximal muscles in upper and lower limb.

	Lower limb (LL)	*Upper limb (UL)*
Distally (disc)	big toe	First Dorsal Interosseous (hand)
Intermediate (intrm)	foot dorsiflexion	wrist dorsiflexion
Proximal (prox)	knee extension	elbow extension

Muscle power is scored according to the categories of the MRC scale except that the scales are inverted in order that normality consistently is scored as 0 in this investigation.

Score	**Criteria**	(MRC scale)
0	normal power	(5)
1	active movement against gravity and resistance	(4)
2	active movement against gravity	(3)
3	active movement, gravity eliminated	(2)
4	trace or flicker of contraction	(1)
5	no contraction	(0)

Reference:

Mayo Clinic & Mayo Foundation. 1991. *Clinical exmination in Neurology.* 6ed. Mosby;

Name: _____ DoB: ___/___/___ Study no: _____

Date: ___/___/___

Investigator: ____

Neuropathy (signs) Record Sheet administered at the INS

	R		L
Cranial Nerves	Yes	No	Yes
No			
III, IV, VI Extra-ocular weakness	.. ..	..	..
V Sensory & motor weakness	.. ..	..	..
VII Facial weakness	.. ..	..	..
X Palate weakness	.. ..	..	..
XII Tongue weakness	.. ..	..	..

			Score		*Key:*
Reflexes (0-2)	Biceps		____		
(right side)	Triceps		____		0: normal
	Supinator		____		1: present only on reinforcement
	Knee		____		2: absent,
	Ankle		____		
		Sum of scores (0 - 10)		____	
Sensation (0-4)	Pin prick		____		0: normal
(right side)	Vibration		____		1: reduced below ankle
	Fine touch		____		2: reduced below knee,
	Position		____		3: reduced in hand and below knee
		Sum of scores (0 - 16)		____	4: reduced below elbow and below knee.

Muscle Power (0-5) (right side)	LL dist (big toe)	____	Inverse MRC scale
	LL intrm (foot dorsiflexion)	____	0: normal power
	LL prox (knee extension)	____	1: active movement against resistance
	UL dist (FDI)	____	2: active movement against gravity
	UL intrm (wrist dorsiflexion)	____	3: active movement, gravity eliminated
	UL prox (elbow extension)	____	4: flicker of contraction
	Sum of scores (0 - 30)	____	5: no contraction

APPENDIX 2

Neurophysiological Protocol

Protocol for nerve conduction studies, EMG and SFEMG

1. Rationale

The techniques employed are well established and validated over decades of routine clinical use in Neurology Departments all over the world[1,2]. It is important to appreciate and eliminate the possible sources of error in the measurements[1(Chapter 7),3] For this reason and because some are invasive, these tests are carried out by trained neurophysiologists in a fully equipped laboratory.

Nerve conduction studies and electromyography (EMG) will be performed by staff qualified in neurophysiology (Dr Abdel-Aziz , Dr Julu or Dr Jamal). Single fibre EMG (SFEMG) will be performed by Dr Jamal because of higher requirement of skill and experience for this test. All investigation will be performed on a Medelec 2 channel or 5 channel EMG machine (Mystro MS 20 or Mystro MS 25). The limb surface temperature will be measured and maintained at 34 °C by heating if required. Where no side is indicated, the right side will be tested unless it is clear from the neuropathy questionnaire that this side is affected asymmetrically. In that case, the left side will be used. A copy of the Nerve Conduction & EMG Record Sheet will be retained at the INS.

Nerve conduction

Nerve conduction studies assesses function in the large diameter nerve fibre population of peripheral nerves. Both motor and sensory functions are investigated in the distal parts of both an upper and a lower limb. Hands and feet are used because a toxic neuropathy is likely to affect the far periphery earlier and more severely.

Motor conduction studies: Surface recording electrodes (Medelec type 16934) are placed over the muscle. The nerve, enervating the muscle is stimulated using surface stimulating electrodes (Medelec type 16893) with short duration electrical current pulses of supra-maximal amplitude to two sites. The latency (delay from stimulus to the first deflection of the muscle action potential) is recorded for both stimulating sites while the peak to peak muscle action potential amplitude from stimulation of the site closest to the muscle is measured. The nerve conduction velocity is calculated as the distance between the stimulation sites divided by the latency difference. The F wave (monosynaptic reflex response) is elicited at a lower stimulus current and its latency measured. The number of times the F wave is elicited during ten consecutive stimulations (F wave persistence) is noted as a percentage.

Outcome variables (for each of the two sites): Latency(distal site), muscle action potential amplitude, nerve conduction velocity, F wave latency and F wave persistence (%)

Sensory conduction studies: Surface recording electrodes (Medelec type 16934) are placed over the nerve at wrist for median nerve and knee for sural nerve. Stimulating electrodes are placed on 1st index finger (digital ring, Medelec type 16639) for median nerve and over the nerve at ankle for sural nerve (Medelec type 16893). The responses to short duration electrical current pulses of supra-maximal amplitude are averaged over eight stimuli. The latency (delay from stimulus to the peak of the nerve action potential) and peak to peak amplitude is measured. The nerve conduction velocity is calculated as the distance between the stimulation and recording sites divided by the latency.

Outcome variables: Latency, amplitude and nerve conduction velocity.

EMG

Concentric needle electromyography is used in this study to detect any neurogenic changes.

Spontaneous activity (fibrillation) is a sign of ongoing denervation of muscle fibres. Long duration and large amplitude motor unit potentials and/or polyphasic motor unit are caused by denervation followed by reinnervation and indicate a chronic loss of motor units (motor nerve axons). A reduced interference pattern is also a sign of chronic loss of motor units.

Needle electromyography is performed in the extensor digitorum brevis, tibialis anterior and extensor digiti communis muscles. A disposable concentric needle electrode (Medelec type NDMC37) is inserted to each of the three muscles in turn. (EMG in the extensor digiti communis muscle is performed to make sure that abnormal SFEMG is not concurrent with neuropathy).

At rest (no muscle contraction) the presence of fibrillations, positive sharp waves, fasciculations and high frequency discharges are noted with a 1 for occasional, 2 for profuse while 0 indicates none. Fibrillations occur in recently denervated muscle.

The range of amplitudes (peak to peak) and duration of motor unit potentials are measured at low voluntary contraction. The percentage of polyphasic units (four phases or more) is also noted. Polyphasia is caused by denervated muscle fibres being reinnervated by collateral sprouting from remaining nerves and is an indication of neuropathy.

At maximum voluntary contraction, the maximum peak to peak amplitude is noted. The density is marked **N**(ormal) if the interference pattern is full, **SR** (slightly reduced) or **R**(educed) otherwise. Stability of motor unit potentials with re-innervation is tested on polyphasic unit using a trigger delay facility. A "yes" indicates instability. The parameters in this paragraph are not used for scoring but are recorded and may be used for profiling.

A score is given for each muscle:

Score	*Ccriteria*
0	</= 15% polyphasic motor units and no spontaneous activity
1	115 - 35% polyphasic motor units and/or spontaneous activity
2	> 35% polyphasic motor units and profuse spontaneous activity

An overall score (sum of scores for individual muscles) is also given.

Outcome variables: Score for each muscle and sum of scores.

SFEMG

The technique of single fibre EMG is also well established and well validated in many Neurophysiology Departments all over the world[4]. It is an extremely sensitive test for study of the neuromuscular junction and peripheral parts of the motor unit[5] It is also very important that a highly trained and experienced neurophysiologist performs this test in a hospital environment. A multicenter study showed little variation of mean jitter values between centres[6] thus allowing published, age corrected confidence limits to be used.

For this study it has been decided to measure stimulated jitter instead of jitter from voluntary activated motor units. The stimulated jitter technique is faster to perform because it is easier for the investigator to place the special needle electrodes near a suitable signal source and it requires much less co-operation from the subject.

In "voluntary" SFEMG, the operator has to locate two potentials from muscle fibres of the same motor unit while the subject contracts the muscle slightly. The mean consecutive difference of the interpotential interval between the two muscle fibre action potentials (jitter) is measured for 100 discharges in ten different muscle fibre pairs.

In "stimulated" SFEMG, a few muscle fibres are activated by electrical stimulation using a monopolar needle placed in the end-plate zone of the muscle. The recording needle electrode is moved until one muscle fibre action potentials, synchronised with the stimulus is found. The subject is just required to relax. Here the jitter is the mean consecutive difference of the interpotential interval between the stimulus and the muscle fibre action potential for 100 stimuli. Stimulated jitter gives the same qualitative information as voluntary jitter and the relationship has been found to be[7]:

$$\text{Stimulated jitter} = (\text{voluntary jitter})/(\text{square root of } 2) + 10\ \%$$

Stimulated single fibre electromyography is performed in the extensor digiti communis muscle. The SFEMG recording electrode with an active electrode diameter of 25 μm (Medelec type 22584) is inserted into the muscle. A monopolar stimulating electrode (Medelec type 53512) is also inserted into the muscle at the end-plate zone. Low current electrical stimuli are used to recruit motor units repetitively. Jitter measurements are performed on sequential potentials from a single muscle fibre in relation to the stimulus and are based on 100 stimulus pulses. Measurement from muscle fibres of ten different motor units are combined.

Outcome variables:

Mean overall jitter	Mean jitter of the ten measurements.
% abnormal jitter	Number (in %) jitter value exceeding the normal limit for a single measurement.
% mean blockings	Overal mean of % blockings (A blocking is the absence of a single fibre potential following stimulation indicating that the muscle fibre did not respond to a nerve impulse)

2. Recording of raw data

General

All measurements (with exception of measurement of distance between stimulating sites) and interpretations are carried out using potentials recorded by the Mystro EMG machine. These potentials are displayed on screen as required and could be available in digital format. When the potentials have been analysed and relevant variables noted on the Nerve Conduction & EMG Record Sheet,the internal stores will be overwritten when the next test is carried out.

Raw data can be stored in two ways; either printed as a screen copy on the EMG machine's inbuilt thermal printer or the digital data can be transferred to a PC. The PC simply acts as a file store; data must be transferred back to the Mystro for viewing and analysis. It is not standard procedure in clinical practice to save all raw data and a requirement to do so for this study prolong the tests and may have cost implications.

Motor nerve conduction

Two potentials are recorded; one from each of the two stimulation sites. Markers are placed at the start of potential deflection and after the distance between stimulus sites has been typed in, the Mystro display latency values and conduction velocity together with the potentials. Amplitude is measured by placing the two cursors on the maximum and minimum points of the potential evoked by stimulation at the distal site.The screens could be printed or the potentials saved in digital form.

Sensory nerve conduction

One potential is recorded as an average of the resonse to between four and sixteen stimuli.One markers remains at the beginning of the potential (start of stimulus) while the other is placed at the peak of the potential deflection and after the distance between stimulating and recording electrodes has been typed in, the Mystro display latency and conduction velocity together with the potential. Amplitude is measured by placing the two cursors on the maximum and minimum points of the potential. The screens could be printed or the potentials saved in digital form.

EMG

The display on the Mystro during the EMG test is free running and only a little data is actually stored. It would be possible keep some samples of any spontaneous activity, motor unit potentials and interference patterns. The screens could be printed or the potentials saved in digital form. However, this will prolong the test considerably and may have cost implications.

SFEMG

For each muscle fibre potential used for jitter measurement, 100 traces are stored before the jitter for that fibre is calculated. At this stage, a sample screen (of say 20 - 50 traces) may be printed or all traces saved in digital form. However, this will prolong the test considerably as a lot of data is generated and will have cost implications.

The jitter values and other parameters for each fibre is saved in a table which also gives the overall mean jitter. This table may be printed or saved to PC in ASCII format.

References:

1. Kimura J. Electrodiagnosis In Diseases of Nerve and Muscle. Principles and Practice. 2nd ed. 1989. F.A. Davis Company, Philidelphia. (Chapter 7: Facts, fallacies and fabcies of nerve stimulation techniques.)
2. Jamal G A, Mann C. (1993) Peripheral nerve and muscle. Current Opnion in Neurology, 6; 724-730.
3. Simpson J A. (1964). Facts and fallacy in rneasurement of conduction velocity in motor nerves. Journal of Neurology, Neurosurgery and Psychiatry, 27:381 - 385.
4. Stålberg E, Trontelj J V (1994). Single fiber electromyography. Studies in healthy and diseasedmuscles. 2nd el. Raven Press, New York.
5. Jamal GA. (1989). Update: Single fibre electromyography: Principles and Applications. Journal of Electrophysiological Technology, 15: 5-16.

SENSORY TEST

Preparation

The tests should take place in a quiet, warm room with subject seated in a comfortable chair. The right foot, after removal of sock and shoe/boot, should rest on a low stool or similar with padded surface. The dorsum (top) of the foot should be kept in a horizontal plane by supporting the foot with a bag filled with rice or sand. The surface temperature of the foot should be measured and if below 31°C, the foot must he heated (e.g. in warm water).

Please follow the summary instructions below. Text in *[italics]* indicates explanations and instructions to the subject.

Thermal sensation test

Explaining the test to the patient is absolutely crucial. The patient must fully understand the test and what is required from him/her. Otherwise the result will be unreliable.

Please take great care of the thermode. It is easily damaged if dropped onto a hard surface.

1. Make sure all connections to **Triple T** are secure. Switch on.

["I am going to test how well you are able to feel small changes in temperature using this machine. The test takes a few minutes, is completely harmless and painless but we need your constant concentration.]

2. Apply the thermode to the top of the foot
   - Thermode surface must be as horizontal as possible
   - The foot should be in a relaxed position

3. Apply the elasticated Velcro strap round thermode and foot
   1. Hold the strap tight without stretching elastic (negligible tension in strap)
   2. Pull the top part of strap so that elastic stretches by 1cm
   3. Press Velcro straps together to fasten (this procedure allows thermode to be applied with a constant and reproducible force).

4. Input patients data (while this takes place thermal equilibrium will develop between thermode and skin)

With display showing: START OR PROGRAM ? Press **P** to enter program mode

Press ← four times until display shows:

UP-DOWN TRANSFORM

2 CHANGES ↑ / ↓

Press ↑ so that 4 changes is indicated

Press **P** to exit program mode.

5. Hand the 'Patient Control Unit' to the subject.

[I am giving you this Control Unit to hold and look at during the test. There are three lights and I will explain how they are used as we go along. First you will see all three lights come on and you may feel the skin under the probe getting warm]

6. Start (press start button) which begins the test with calibration. (Before starting, make sure of thermode application and position, step 3).

If calibration is done inappropriately, then the whole test is unreliable and excessively prolonged. Calibration uses a temperature change of about 2 °C. This may give an idea about whether the threshold is below or above this value.

7. Press ↵ button to start manual Test : The idea is to bracket the threshold between two large values.

[This time you will see light 1 come on alone] Ask subject after the light disappears if he/she could feel the probe becoming warm for a brief period.

Spend as much time as necessary on this. Repeat several times and at least 2 - 3 times at the level that you decide to start the test at. This will be the lowest level which the subject reliably can feel. Level of manual stimulation can be altered by using the ↑ and ↓ keys.
Please note that the **UDTR** (Up and Down Transform Rule) test will start at the last manual stimulation value.

[From now on the test will be slightly different.

The green light will come on first to alert you to that a test is about to start. Next light 1 will go on and stay on for a short time and then go off again. After a short pause, light 2 will come on and then go out again. Last, the green light will disappear. During one of these two periods indicated by light 1 and 2 - and only during one of them - you may feel a little warmth. I would like you to tell me, when all the lights have gone off, with which one, 1 or 2, you felt this warmth. You may sometimes find difficulty in deciding because some of the temperature changes are small, but you still have to indicate a number to the best of your judgement.

You will have several such trials and at the end of each we would like you to tell us one of the two numbers you think you felt the warmth with. Please keep concentrating on the unit in your hand all the time.]

8. Press start button to proceed with the UDTR test.
   - Subject *must* answer 1 or 2
   - Subject 's reply *must* be keyed in by the OPERATOR through the keyboard (and not by the subject through the Patient Control Unit)

16. Remove the thermode and **return it to a safe storage place** e.g. the instrument case

Vibration sensation test

1. Make sure all connections to the VIBRAMETER are secure. Switch the power switch at the back of the control unit to ON. On the front panel press the button marked **I** and the digital display will be illuminated.

2. Hold the vibrator in your right hand and let the probe rest on a flat surface. Press with your left hand simultaneously the two buttons marked **C** and **W** . Note that the red dot on the pressure indicator (slit display) moves to the centre. After this happens release buttons **C** and **W**.

["I am going to test how well you are able to feel small levels of vibration using this machine. The test takes a few minutes, is completely harmless and painless but we need your constant concentration.]

3. The vibration amplitude of the probe is controlled using the push buttons marked with arrows or with the thumbwheel. The ↑ button indicates increase and the ↓ button indicates decrease. A fat arrow indicates a rapid change in vibration amplitude while a thin arrow means a slower rate of change. Pressing the **H** button will hold the measured value for reading later. The **R** button toggles the full scale display range of VIBRAMETER between 39.99μm and 399.9μm.

4. As examiner, you should sit in strain free position with comfortable access to, and visual contact with both the test site and VIBRAMETER. During measurements place the probe perpendicular to the test site to provide smooth, painless contact. The test site is 1st metatarsal bone (on top of the foot in line with the big toe) of the right foot. There should be no tendons between the probe and the bone. This placement should be checked during measurement to verify that the vibrator stays in the correct position.

5. Adjust the pressure for centre indication on the VIBRAMETER pressure indicator

6. Start with no vibration and tell the subject that *[this is the feeling of the pressure from the vibrator]*.

Then increase the vibration amplitude until the patient clearly feels a vibration. When these two initial sensation are defined (repeat if thought necessary) you have a coarse indication of the subjects threshold and the subject starts to be come familiar with the measurement procedure.

7. Reduce the vibration amplitude so that the subject cannot feel the vibration. Ask the subject *[please say "yes" when you feel the vibration again]* and increase the vibration amplitude at the slower rate (using the thin ↑ button). Remember the meter reading at which the subject said "yes". Now ask the subject *[please say "off" as soon as you no longer feel the vibration]* Decrease the amplitude (at the slower rate) until the subject reports "off" and remember the meter reading. Write

the "yes" and "off" values into the appropriate columns on the Sensory Test Record Sheet and calculate the average of these two value and enter it in the third column.

8. Repeat the procedure in point 6 until three consecutive mean values with less than 10% variation are obtained

9. Enter the mean of last three mean threshold values as the Vibration Threshold on the Sensory Test Record Sheet.

APPENDIX 3

Neurological Score Sheets

Name: _____ DoB: ___/___/___ Study no: _____

Date: ___/___/___

Investigator: ____

Sensory Test Record Sheet administered at the INS

Thermal Sensation Test

Dorsum right foot

Heat threshold ____ °C

Cold threshold ____ °C

Vibration Sensation Test (Somedic)

(Vibration threshold = average of last three means showing < 10 % variation)

Right 1st metatarsal bone		
"Yes" Level	*"No" Level*	*Test mean* µm
Vibration threshold		

Right index metacarpal bone		
"Yes" Level	*"No" Level*	*Test mean* µm
Vibration threshold		

Vibration threshold (GVS)

Right 1st metatarsal bone _____ µm (File name _____)

Right index metacarpal bone _____ µm (File name _____)

Name: _____ DoB: ___/___/___ Study no: _____

Date: ___/___/___

Investigator: ____

Nerve Conduction & EMG Record Sheet administered at the INS

Nerve conduction

(Right side)	*Com. Per. nerve*		*Median nerve*		*Sural*	*Median*
	Motor		*Motor*		*Sensory*	*Sensory*
Stimulation site	Ankle	Knee	Wrist	Elbow	Ankle	Finger
Latency [ms]						
Amplitude	mV		mV		µV	µV
Conduction distance [cm]						
Cond. velocity [m/s]						
F wave latency						
F wave persistence (%)						

EMG

*Needle No:*_____

		Right Ext Dig Brev	*Right Tib Ant*	*Right Ext Dig Com*	
Spontaneous activity	*Fibril*				
	Pw/Atrof				
	Fasc				
	HFD				
Motor unit potentials	*Amp*				
	Dur				
	% Polyph				
Interference pattern	*Density*				
	Amp				Sum of scores (0-6)
Stability	*(yes - no)*				
Score					

(Score
0 0 - 15% polyphasic and no spontaneous activity
1 15 - 35% polyphasic and/or spontaneous activity
2 >35% polyphasic and prosuse spontaneous activity

(Score
0 0 - 15% polyphasic and no spontaneous activity
1 15 - 35% polyphasic and/or spontaneous activity
2 >35% polyphasic and prosuse spontaneous activity

SFEMG (Stimulated, from 10 muscle fibres) Investigator: ____

Right Ext Dig Com

Mean overall jitter _____ μs
% abnormal jitter _____ %
% mean blockings _____ %

APPENDIX 4

Validation of Sensory Tests

Choice of percentiles defining abnormal tests

In all three sensory tests, a cut-off point is needed above or below which is then defined as 'abnormal'. All three tests give thresholds estimates on continuous scales, and the cut-off point chosen will determine the sensitivity and specificity of each tests.

Sensitivity in this case is defined as the probability of being 'abnormal' on the test out of all those who truly have peripheral neuropathy (defined clinically), in other words, true positives. Similarly, specificity is the probability of being negative (or 'normal') out of all those who are truly not clinical cases, that is, true negative.

In the table below sensitivity = a/(a + b) x 100; while
specificity = d/(c + d) x 100.

Note that 100 - sensitivity = false negative percentage and
100 - specificity = false positive percentage.

	Test Abnormal	Test Normal	Total
Clinically a case	**a**	b	a + b
Clinically not a case	c	**d**	c + d

A high cut-off point will give good specificity and lower sensitivity, while a low cut-off point will give good sensitivity but reduce specificity. The cut-off point should be based on the general or 'normal' population to avoid bias.

If the prevalence of probable neuropathy is around 0.5% to 1% then it seems logical to go for a 99th percentile (this will be at the lower or upper end of the distribution depending on which side abnormality lies). However, this may be too high giving very poor specificity, so that the 95th percentile may be better.

The threshold values for all three tests increase with age so that the cut-off point will also increase with age. Figures 1 and 2 show plots of data from a sample of 68 healthy men and women aged 16 to 76 years from Glasgow for the hot and cold thermal tests. The regression line was obtained from regressing the natural logarithm of the thresholds on age, as a log linear relationship was found to be a better fit to the data than a simple linear model. The sample was composed of 47% females and 53% males, and although males had significantly higher thresholds than females, the slopes of the regression line did not differ significantly between males and females, and so only one line is presented in figures 1 and 2 (Appendix 4). The residuals from the regression model were obtained (observed values minus the fitted values) and the 95th percentile value was calculated. This constant value was added to the expected value for each age based on the regression model. Finally, the 95th percentile line and fitted line were converted back to the original scale by exponentiating these lines to give figures 1 and 2. This procedure corresponds to that given by Dyck et al (1985) to obtain percentile lines. For use in the field, tables will also be produced giving the mean threshold value for each age and the associated 95th percentile.

Normal values of the vibrameter threshold have been obtained from a sample of 110 healthy male volunteers, aged from 10 to 74 years, with no signs or symptoms of neurological diseases (Goldberg and Lindblom 1979), which is included in the vibrameter manual (Somedic IV). The plot of vibration threshold against age is shown in figure 3 and the 95th percentile inserted. A table

showing the mean at each age with the associated 95 percentile limit will also be available when carrying out this test in the field.

APPENDIX 5

Neuropsychological Protocol

NEUROLOGICAL HEALTH OF SHEEP DIPPERS
NEUROPSYCHOLOGICAL TEST PROTOCOL

Personnel

Neuropsychological testing will be carried out by Dr Ruth Gilham, or an Assistant Psychologist, (still to be appointed), or other personnel involved in the project who will have been briefed and trained in the use of the tests by Ruth Gilham.

Timing

Subjects will attend for neuropsychological testing on the same day that neurophysiological investigations are to be performed. Two and a half hours should be allocated to neuropsychological testing.

Set-up

The Cambridge Neuropsychological Test Automated Battery (CANTAB) will be administered by computer with a touch sensitive screen. Verbal instructions and supervision of the subject are necessary during administration. All subjects should be tested in the same room, with the computer set up on a table with a height adjustable chair.

Tests not administered by computer, (National Adult Reading Test, Anxiety and Depression self-rating scales) will be administered by the same investigator on the same occasion, immediately after the automated test battery.

Test Description and Scoring

Tests followed by (C) are CANTAB tests. All CANTAB tests are administered by computer, using a touch sensitive screen. Instructions for test administration, and instructions to subjects, for the CANTAB are supplied with the software. Test scores are in the form of response time in milliseconds, and, if applicable, number of items correct. More detailed analysis of performance variance, error type etc. is available.

Instructions for subjects are printed on the self-rating scales.

ATTENTION:

Motor Screening (C):

A cross appears on the screen. The subject is required to touch it as quickly as possible. Response time is recorded.
Score: Mean reaction time

Reaction Time (C):

The test is divided into five sections.

1. Pointing to a single stimulus;

2. Five choice reaction time;

3. Time to release touch pad following stimulus;

4. Time to release touch pad and point to stimulus;

5. Five choice release and point;

Score: Mean reaction time, or decision time and movement time

Matching to Sample Visual Search (C):

An abstract pattern is displayed in the middle of the screen. Then 1, 2, 4 or 8 alternative patterns are displayed. The subject has to touch the one that matches the sample.

Score: Total correct. Mean latency (msec).

MEMORY:

Pattern Recognition (C):

A series of twelve visual patterns are presented, followed by twelve pairs of patterns, one of which was previously presented. The subject must select the pattern previously seen.

Score: Total correct. Mean latency (msec).

Spatial Recognition (C):

A white square moves to five different places on the screen. Pairs of white squares are presented and the subject must select the one in the position previously seen.

Score: Total correct. Mean latency (msec).

Paired Associate Learning (C):

The subject is required to remember patterns associated with different locations on the screen. ix boxes are shown and a pattern revealed in any number of them from 1 to 6, a different pattern in each case. Then each pattern is shown centre screen, and the subject must point to the location at which it was previously revealed.

Score: Total number correct on first presentation.

Spatial Span (C):

A pattern of white squares is displayed which change to red and then back to white in specific sequences. The subject must touch each square in the order in which it changed colour.

Score: Length of longest sequence subject could recall.

RAVLT:

The Rey Auditory Verbal Learning Test is a list of fifteen common nouns read to the subject by the investigator. The subject is asked to recall as many as he/she is able. The list is read five times. Score is total numbers of words recalled out of a possible total of 75.

Score: Number correct out of 75.

PSYCHOLOGICAL STATE

Hospital Anxiety and Depression Scale (HAD), and General Health Questionnaire (GHQ) are both self-rating questionnaires.

Score: GHQ - max. 28
HAD - Anxiety score, Depression score, both max. 21.

CONTROL VARIABLE

NART:

The National Adult Reading Test is presented as a list of fifty words printed on a card. No word is pronounced phonetically (eg. Chord). The subject is asked to read the words aloud.

Score: Number of errors out of a possible total of 50, converted to IQ estimate.

APPENDIX 6

Neuropsychological Score Sheet

Neuropsychology Data Recording Form

Draft (20Aug 97)

0. Identification

Study Number		[ ] [ ]
Individual's name		[ ]
Sex	*M/F*	[ ]
Date of Birth	*DDMMYY*	[ ]
Date of Survey	*DDMMYY*	[ ]
Start Time of Survey	24hr	[ ]
Technician's Initials		[ ]

1. Profile

National Adult Reading Test - Errors	(0 - 50)	[ ]
Full Scale IQ		[ ]
Rey Auditory Verbal Learning Total Words Recalled	(0 - 75)	[ ]
GHQ Somatic Symptoms	(0 - 7)	[ ]
Anxiety and Insomnia	(0 - 7)	[ ]
Social Dysfunction	(0 - 7)	[ ]

Severe Depression	(0 - 7)	
TOTAL		

HAD		
Anxiety	(0 - 21)	
Depression	(0 - 21)	

CANTAB

2. Attention

Motor Screening
Mean Latency (msec) | | | |

Reaction Time
Set 1 Reaction Latency | | (msec)
Set 2 Reaction Latency | (msec)
Set 3 Reaction Latency | (msec)
Set 4 Movement Latency (msec)
Set 5 Movement Latency (msec)

Visual Search
Total correct | (0 - 50)
Reaction Time (msec) | | | |
Movement Time (msec) | | | |

3. Memory

Pattern Recognition | Total correct (0 - 20)
Mean Latency (msec) | | | |

Spatial Recognition | Total correct (0 - 20)
Mean Latency (msec) | | | |

Paired Associate Learning Total first trials memory score | (0 - 20)

Spatial Span longest sequence

APPENDIX 7

Interval Since Farm Visit Questionnaire and Protocol

Protocol for the completion of the exposures since farm or factory visit questionnaire

Introduction

This questionnaire has been designed for use in the third phase of the project, the field survey, and will be used prior to neurological and neuropsychological assessments. The purpose of the questionnaire is to obtain information on relevant exposures which have occurred since the visit of the IOM survey team during the second phase of the project and exposure history information based on those groups of factors from the revised OP uptake model, which are thought to influence uptake of OPs. These factors include: job history; work with dip wash and work with concentrate; and use of OPs in non-dipping activities.

General Instructions

The subject's personal details should be recorded prior to administering the questionnaire. The actual printed wording should be used for each question. Repeating the question can be helpful where the subject appears unsure about the response required. In certain cases, additional explanatory information is provided within the protocol.

The interviewer should take this opportunity to stress that no information relevant to job or previous occupational exposure should be given to the INS team performing the neurological or neuropsychological assessments.

Comments on Individual Questions

General Information

(a)Farm or factory number Insert the number that has been assigned to this farm. Each box should be filled eg. farm 342 should be recorded as 0342.
(b)Individual's name and code Insert the individual's name and corresponding code number. At some sites father and son may have the same name, under these circumstances record senior and junior as appropriate.
(c)Male or female Insert M or F in space provided.
(d)Date of Birth Each box should be filled eg. 7 August 1952 should be recorded as 070852.
(e)Date of Assessment Each box should be filled as described above.

Question 1 Tasks performed since our visit

Read out the list of options and mark Y or N in the box opposite each of the answer options. Each of these tasks may be associated with the use of pesticides either organophosphates or pyrethroids.

If the subject answers NO to all of the options in question 1 proceed to question 7

If the subject answers YES to any of the options proceed to question 2

Question 2 When did you last carry out these tasks?

If the subject has performed several of the tasks listed in question 1, ask them which task they performed most recently. Then ask them when they performed this task and record the date (ensuring that each box is filled eg. 7th July 1997 should be recorded as 070797).

Question 3 Accidents or incidents with pesticide products

Ask the subject if they have had any accidents associated with sheep dip or pour on products - again these may be pyrethroids or organophosphates

Accidents may include spillage onto body parts of concentrated dip products, or heavy soaking with dilute dip for example during dipping or as a result of falling into the dip bath. Record the nature of any accidents/incidents.

Record the date of this accident. (If more than one accident has occurred since the survey team visit, record the date of the most recent incident).

Question 4
Did you handle concentrated dip or pour on **during tasks listed in Question 1?**

Define concentrate as sheep dip when it is not diluted by water.
This question again relates to the time since the IOM survey team visit.
Mark Y or N in the box provided.

Question 5 Handling concenntrated pesticide products

If the subject has handled concentrate substances record the most recent date that they handled these substances. Products include both organophosphates (eg. for dipping) and pyrethroids (eg. as pour-ons).

Question 6 Use of gloves.

Ask the subject whether they usually wear protective gloves when pouring out concentrate or adding it to the bath.

Mark Y or N in the box provided.

Question 7 Other uses of pesticides

Ask whether pesticides have been used for any other purpose other than the tasks listed in question 1 for example crop spraying, treatment of stored grain, use on domestic pets, wood treatment or in the garden.

Record the nature of the task and the substance used (if known). Try to establish whether this was organophosphate based or not. Record the date when the most recent task was performed using this substance.

Question 8	Have you worked regularly with any of the following since our visit?
Vibrating tools	eg.driving tractors, use of chain saws, pneumatic drills
Lead based paints	eg.chipping/burning lead-painted surfaces, pigments
Solvents	eg.thinners, degreasers, paints, varnishes

Enter Y or N in the appropriate box.

Questionnaire to Determine Relevant Exposures Since Farm Visit

IOM (30/10/97)

Subject Code N | | | | | |

Individual's name

Sex *M/F*

Date of Birth *DDMMYY* | |

Date of Assessment *DDMMYY* | |

The following questions are about the work you have been doing since our visit to your farm. It is important that you tell us all about this work because it may affect the assessment we are doing today.

1.Which of the following tasks have you performed since our last visit to your farm?
(Enter Y/N for each task)

Dipping

Cleaning or emptying the dipping bath

Application of a pour-on

Showering sheep

Treatment of infested sheep

Other use of dips and pour-ons (specify)

☜ *If no to all of these go on to question 7, if yes continue.*

2.When did you last carry out any of these tasks? *DDMMYY* | |

3.Have you been involved in any accidents or incidents with sheep dip or pour-on whilst carrying out these tasks? Y/N

If yes record what happened. Free text.

3.bWhen did this last happen? *DDMMYY* |

4.Did you ever handle concentrated dip or pour-on whilst doing these tasks? Y/N

☞ *If no go on to question 7, if yes continue*

5.When did you last handle concentrated substances? *DDMMYY* |

6.Did you usually wear protective gloves when handling concentrated substances? Y/N

7.Since our visit have you applied any other insecticides on the farm or in your home?

Y/N

If Yes *task*	*proprietary name(s) of substance*	*type*

7.bWhen did you last do this? *DDMMYY* | |

8. Have you worked regularly with any of the following since our visit to your farm? Y/N

Vibrating Tools		
Lead based paints		
Solvents		

APPENDIX 8

Letters of Invitation

September 1997

Dear

Re:Survey of Sheep Dippers Health

Thank you for participating in this survey which took place between October 1996 and May 1997.

The results of the tests suggest that your feet are a little less sensitive to changes in temperature and vibration than would be expected for your age group. Whilst these are borderline results and may be of no clinical significance, we would like to follow them up and would like to invite you to take part in a more detailed assessment by a doctor at The Institute of Neurological Sciences (INS) in Glasgow.

The assessment includes questionnaires, and a number of simple tests similar to those performed during the farm visit, tests of muscle function, and some tests which can detect more subtle health effects. You will not be asked to give a blood sample.

If you are able to take part you will be paid travelling expenses and an allowance to cover time lost at work. We would be most grateful for your assistance with the next phase of the survey, as this is an important area of research which potentially has implications for both farmers and other pesticide users.

If you are willing to participate please complete the enclosed consent form, and return it to the IOM in the envelope provided.

We will contact you in the near future to discuss arrangements for the visit.

We have sent details of results to your GP, and in the usual way if you have concerns about your health you may wish to discuss this with your GP.

Yours sincerely

Dr Adele Pilkington
Occupational Physician

September 1997

Dear

Re:Survey of Sheep Dippers Health

Thank you for participating in this survey which took place between October 1996 and May 1997.

As you may remember, you reported some symptoms on the questionnaire you completed. These symptoms could arise for a number of reasons and do not necessarily suggest an underlying disease. However, we would like to follow you up and would like to invite you to take part in a more detailed assessment by a doctor at The Institute of Neurological Sciences (INS) in Glasgow.

The assessment includes questionnaires, and a number of simple tests similar to those performed during the farm visit, tests of muscle function, and some tests which can detect more subtle health effects. You will not be asked to give a blood sample.

If you are able to take part you will be paid travelling expenses and an allowance to cover time lost at work. We would be most grateful for your assistance with the next phase of the survey, as this is an important area of research which potentially has implications for both farmers and other pesticide users.

If you are willing to participate please complete the enclosed consent form, and return it to the IOM in the envelope provided.

We will contact you in the near future to discuss arrangements for the visit.

We have sent details of results to your GP, and in the usual way if you have concerns about your health you may wish to discuss this with your GP.

Yours sincerely

Dr Adele Pilkington
Occupational Physician

APPENDIX 9

Non-response Record Sheet

Farm Number:

SUBJECT RECRUITMENT TO EPIDEMIOLOGICAL STUDY OF SHEEP DIPPING

Letter:	Subject' Reply: **INF NO YES**	Recruited? **NO YES**

Subject's Name	
Subject's Address	
Phone Number	

Summary of Phone Conversations:

Date	Contact	Summary	Initials

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