COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF PARKINSON'S DISEASE AND PARKINSONISM

INTRODUCTION

1. The COT 1999 report did not specifically consider Parkinson's Disease (PD) as an outcome. In this review, ten studies related to PD. One case-control study considered chronic effects of acute exposure, while the remaining nine considered chronic low-level exposure to OPs and its possible association with the development of PD.

2. PD is an idiopathic degenerative disorder of the central nervous system. It presents clinically as parkinsonian syndrome, with the main symptoms of chronic progressive tremor, bradykinesia (a slowed ability to start and continue movements), rigidity and postural instability (Brown et al 2006). The main pathological feature of PD is a progressive loss of dopaminergic neurons in the substantia nigra of the brain, but the first clinical signs of PD only become apparent after loss of about 70-80% of these neurons. The diagnosis of PD is entirely clinical, although the only definitive confirmation of a diagnosis would be histopathology on autopsy; none of the studies in this review contain such information.

3. The mean age of onset of PD is between 60 to 65 years (Brown et al 2006). Age is associated with increasing risk of PD, but the underlying process of PD is distinct from the natural aging process (Goldman and Tanner 1998). Genetic factors can influence the risk of PD, and it has been found that there are higher rates of PD in relatives of sufferers (Foltynie et al 2002). A number of external factors can also produce parkinsonism similar to that of idiopathic PD, including head injuries, repeated head trauma or repeated loss of consciousness, and neuroleptic (tranquiliser) drugs (Adler 1999). In 1983, it was observed that a toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), led to the development of acute parkinsonism, similar in nearly all clinical, pathological, and biochemical aspects to the idiopathic disease, in a small group of drug addicts in northern California (Langston et al 1983). This led to an interest in the role of environmental toxins in the development of PD and parkinsonism (Dick et al 2007), and especially to an interest in pesticides, since there is structural similarity of a metabolite of MPTP, 1-methyl-4-phenylpyridine (MPP+), to the herbicide paraquat (IEH 2005). Numerous epidemiological and toxicological studies have since then considered pesticides as a risk factor for PD and parkinsonism, although conflicting results have been presented (IEH 2005).

4. It is important to establish a diagnosis of PD. This is based entirely on clinical examination. The idiopathic disease has to be distinguished from other possible causes of parkinsonian syndrome, such as those outlined above. Clinical diagnostic

criteria which are widely accepted have been developed by the UK Parkinson's Disease Society Brain Bank, and are described in the IEH 2005 report. The diagnosis is a three-step process.

5. Step 1 is to make a diagnosis of a parkinsonian syndrome. There are 4 cardinal signs of parkinsonism: resting tremor, rigidity, bradykinesia, and impaired postural reflexes, which are usually seen late in the development of the disease. For a diagnosis of PD, bradykinesia must be present, and at least one of the other 3 symptoms. Bradykinesia is the core disabling feature of parkinsonism, involving difficulty in initiating and maintaining movement and a loss of automatic movements. Tremor is the most obvious clinical sign of PD, and must be present at rest, distinguishing PD from the condition of essential tremor, in which tremor is present on voluntary movement. Rigidity of the muscles is characteristic of PD, and postural instability leads to unsteadiness or lack of balance.

6. The second step of diagnosis involves the exclusion of specific causes of parkinsonian syndrome, such as a history of repeated strokes or of repeated head injury, or the presence of a brain tumour. The third step involves identifying supportive prospective positive criteria for PD, such as unilateral onset, tremor present at rest, the progressive nature of the disorder, or an excellent response to L-dopa. The details of how diagnosis was made were noted for the ten studies in this review in Table 9 of this section.

FINDINGS OF THE IEH REPORT AND THE GEOPARKINSON STUDY

7. The IEH 2005 systematic review identified over 5000 references, and the authors considered over 300 papers in detail. Their conclusion was that, "*there does appear to be evidence of a potential role of pesticides in the development of PD*". The Geoparkinson study, described in Dick et al 2007, investigated the associations between Parkinson's disease and other degenerative parkinsonian syndromes and environmental factors in five European countries. The authors also came to the conclusion that there was evidence to suggest the risk of PD was associated with pesticide use. Both studies, however, found the association with pesticides in general, and specific pesticides were not considered. The ten studies considered in this review provided evidence that was specific to OPs as a chemical group, or to particular OPs.

ACUTE EXPOSURE WITH CHRONIC EFFECTS

Case-control Study in India

8. Only one study investigated the possible chronic effects of acute exposure to OPs. Das et al 2011 considered 345 cases of idiopathic PD attending the neurology department of Burdwan Medical College and Hospital. Diagnosis was confirmed by a neurologist, based on the UK Brain Bank criteria, and cases were matched with 370 controls who were sex-matched and age-matched ±3 years. A number of factors were included and analysed separately as potential confounders, including smoking habits and coffee and tea intake. However, the limitation of the study was

that only 8 subjects and 2 controls had a history of acute poisoning with OPs (Annex 9, Table 7.A.2, Das 2011, "Numbers of cases and controls with acute OP poisoning"). Although a *p* value of 0.046 suggesting possible significance was obtained (Annex 9, Table 7.A.2, Das 2011, "Estimated coefficient value in logistic regression equation"), the numbers involved were too small to draw any conclusions on the evidence.

EFFECTS OF CHRONIC LOW-LEVEL EXPOSURE

US Cohort Study of Chlorpyrifos Production Workers

9. Albers et al 2004b is a cohort study conducted in the USA by the Dow Chemical Company in Midland, Michigan. The study evaluated 2 groups of chemical workers at the Company on 2 occasions, one at baseline and the other 1 year later. Fifty-three employees who were engaged in the manufacture of chlorpyrifos took part at baseline and 1 year follow-up; 58 employees engaged in manufacture of Saran plastic film, with no known exposure to neurotoxicants, were used as a reference group, and 60 participated at baseline, with 58 also participating at 1 year follow-up. This carefully designed study included measurements of urinary excretion of OP metabolites as a measure of exposure, and estimates of air levels of chlorpyrifos from personal air sampling data. The purpose of the neurological examinations performed was to evaluate extrapyramidal signs and to categorise subjects as having possible, or probable, extrapyramidal dysfunction; diagnosis of PD was not attempted. Examinations were undertaken by a board-certified neurologist, and used the motor components of the validated Unified Parkinson's Disease Rating Scale. No evidence of parkinsonism was found in this study (Annex 9, Table 7.B.1, Albers 2004b, "Clinical examination results in chlorpyrifos production workers").

US Cohort Study of Occupationally Exposed Men in Washington State

10. Engel et al 2001 is a study of a cohort of men in Washington State occupationally exposed to pesticides. Occupations included orchardists, professional pesticide applicators and other farm or agricultural workers. Subjects belonged to a cohort established in the 1970s, before most of them were likely to show signs of parkinsonism, which minimises selection bias. A total of 238 subjects were studied for self-reported exposure to any OPs, and to the specific substances azinphos methyl, diazinon and methyl parathion. Subjects were grouped into tertiles, and prevalence ratios for the participants were calculated. The results of the study did not suggest an increased prevalence of parkinsonism associated with exposure to OPs, or with exposure to the three specific OPs (Annex 9, Table 7.B.1, Engel 2001, "Parkinsonism prevalence ratios by exposure related to pesticides"). Limitations of the study were the incomplete follow-up of the original cohort, as only 56.9% of the subjects were contacted, and only 67% of those agreed to participate.

US Study of PD in The Agricultural Health Study Cohort

11. Kamel et al 2007a is an investigation of exposure to pesticides, and to 10 specific OPs, in the Agricultural Health Study based in Iowa and North Carolina. A total of 83 participants reported physician-diagnosed PD at enrolment in 1993-1997,

defined as prevalent cases, and 78 reported physician-diagnosed PD at follow-up in 1999-2003, defined as incident cases. Large numbers of cohort members who did not report PD were available as a comparison population: more than 79,000 at enrolment, and more than 55,000 at follow-up. The study did find an association between overall pesticide use and incident PD for subjects in the highest quartile of cumulative days of pesticide use against the lowest, but the results for specific OPs did not suggest a significant association with the development of PD (Annex 9, Table 7.B.1, Kamel 2007a, "Prevalent and incident PD in Agricultural Health Study participants who used specific OP pesticides").

French Case-Control Study of Agricultural Workers

12. Elbaz et al 2009 is a French study of 224 cases of occupationally exposed workers who applied for healthcare for PD to a health insurance covering workers in agriculture. Information on use and frequency of use of pesticides was self-reported, although interviewers checked sources of information about the chemicals that subjects used, including old pesticide containers on the farms where subjects worked, and reviewed bills and farming calendars, so that exposure information was not based entirely on subjects' recall. The study found a significant association between professional pesticide use and PD, and a significant association with use of insecticides and PD for men, though not for women. The association with the specific category of OPs was not found to be significant (Annex 9, Table 7.B.2, Elbaz 2009, "Results for pesticide and insecticide exposure", and "Results for exposure to OPs"). This was a study with careful matching of a large number of controls (557), matched on sex, age ±2years and health insurance affiliation office, and detailed analysis to adjust for confounders, including stratification of analyses by sex, median age at onset and median disease duration, and adjustments for cigarette smoking and the results of the Mini Mental State Examination, a test used to assess cognitive impairment.

US Case-Control Studies of Effects of Chronic Low-Level Exposure

13. Firestone et al 2005 is a study of 250 newly diagnosed PD case patients, compared to 388 randomly selected subjects from the same Group Health Cooperative in western Washington State. Thus, the study was population-based. The controls were matched by sex, age in 10-year categories, location of the Group Health Cooperative (GHC) at which they were enrolled, and by original year of enrolment at their GHC. Diagnosis of PD was carried out rigorously, using the 4 cardinal signs of parkinsonism, exclusion of other possible causes of parkinsonism, and a combination of other measures, such as use of databases with diagnostic coding and the use of pharmacy information, to ensure complete ascertainment. Diagnosis was further confirmed by review of patients' medical charts by a neurologist. Exposure, on the other hand, was not clearly determined, with the use of occupational titles as an estimate of occupational exposure, as well as self-reported use. Home-based exposure was self-reported. No significant associations were found between PD and exposure to OPs occupationally or in the home (Annex 9, Table 7.B.2, Firestone 2005).

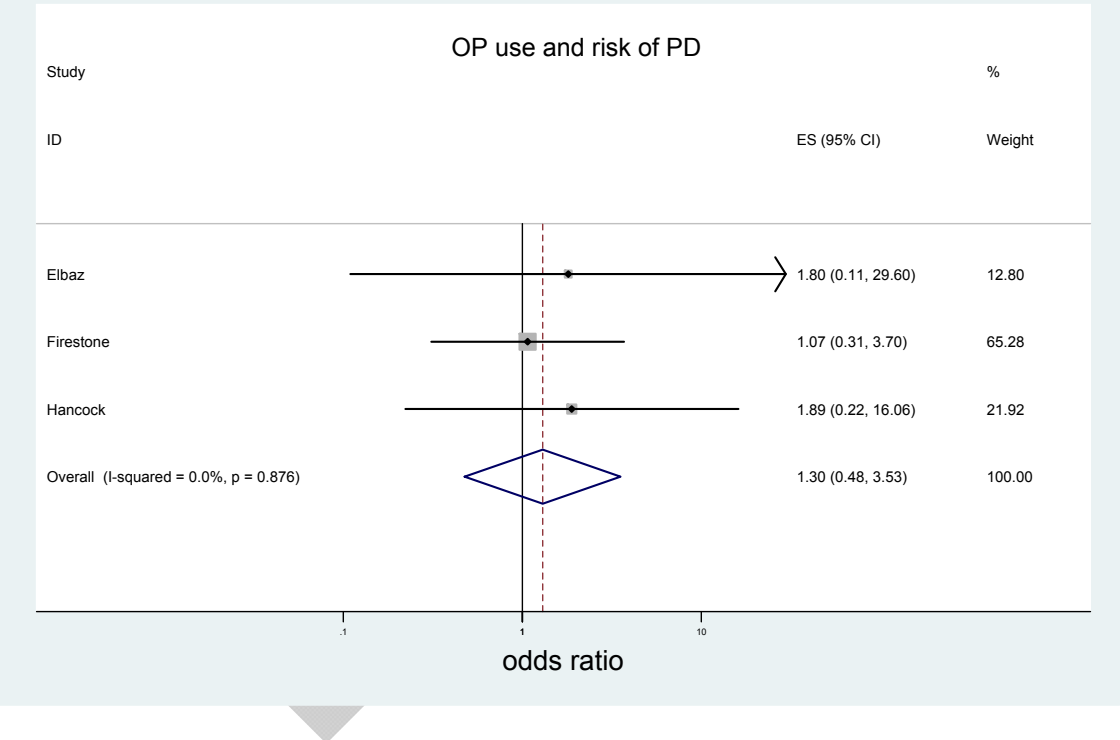
14. Gatto et al 2009 is a population-based case-control study including 368 cases, conducted as part of the University of California Los Angeles (UCLA)

Parkinson's Environment and Genes Study. Diagnosis of PD was made on the strength of the subject having at least two of the 4 cardinal signs of parkinsonism, and no suggestion of parkinsonian syndrome due to other causes, or any other atypical features presenting in the patient, such as cerebellar or pyramidal signs. Confirmation of the diagnosis was made by a UCLA movement disorder specialist. A geographic information system (GIS)-based model was used to estimate potential contamination of well water from agricultural pesticides. The authors reported on specific OPs and results seemed to suggest a role of some substances in the aetiology of PD: for the relative risk of PD from potential exposure to diazinon in well water OR=1.58, 95% CI 1.03-2.43, but results for chlorpyrifos and dimethoate did not suggest increased risk (Annex 9, Table 7.B.2, Gatto 2009, "Relative risk of PD from potential exposure to individual OP pesticides in well water"). The relative risk of PD from combined potential inhalation and ingestion of the OPs diazinon, dimethoate or chlorpyrifos appeared to be significant for all three substances (Annex 9, Table 7.B.2, Gatto 2009, "Relative risk of PD from potential inhalation and ingestion of OP pesticides"). However, the GIS-based model, although it used reports of pesticide application rates within 500 metres of each subject's home, can only provide a general estimate of exposure for each subject, not specific data. Furthermore, well water was not sampled to make direct measurements of the specific OPs, so exposure was estimated, not measured directly. It is also likely that subjects were exposed to a number of pesticides, since several of them were co-applied, and well water in rural areas might be contaminated with multiple pesticides and possibly industrial agents and metals as well, so the effect measures for individual substances were not as precise as they appear.

15. Hancock et al 2008 is a family-based case-control study, with 319 cases drawn from 308 families, and 296 controls, 252 of whom were relatives, and the remaining 44 were either spouse or other unrelated controls. Diagnosis of PD was made on the strength of the subject having at least two of the 4 cardinal signs of parkinsonism, and a clinical examination by a board-certified neurologist using the validated full Unified PD Rating Scale. Other possible causes of parkinsonism were considered, and individuals with an unclear diagnosis were excluded from the analyses. Exposure, however, was self-reported for all use, including application at work, at home, or in the garden. Furthermore, most of the information in the paper related to pesticides as a group, and there was only one measure for Ever use of OP insecticide and an association with PD, which has an OR=1.89, 95% CI 1.11-3.25 (Annex 9, Table 7.B.2, Hancock 2008, "Ever use of OP insecticide and percentage of exposed cases and controls"). The family-based nature of the study has certain strengths, however. It is possible that exposures may be similar between cases and controls, and there is less likely to be confounding by unmeasured genetic and environmental influences. The study contained adjustment for several possible confounders, including sex, age at examination and cigarette smoking. On the other hand, the limitations of a family-based design are that the cases may not be representative of the general population of PD cases, and the results therefore may not generalised. It also offers less power than a population-based study, because of possible overmatching on exposure history. Thus, although the study reports a significant OR for an association between exposure to OPs and PD, the amount of data was limited, and the study design also has inherent limitations.

16. Since three of the case-control studies were comparable in study design and exposure, it was decided to perform a meta-analysis of these results, to see if a pooled Odds Ratio could suggest significance or otherwise of the results. Three case-control studies which reported ORs for exposure through use of OP pesticides were Elbaz et al 2009, Firestone et al 2005 and Hancock et al 2008. The table below indicates the effect measures that could be compared from the studies, and the figure below shows a forest plot performed using STATA version 11.3 of these results:

Study	Disease	Exposure	Effect measure	Lower limit	Upper limit
Elbaz (2009)	PD	Occupational exposure to OPs (men)	OR 1.80	0.90	3.70
Firestone (2005)	PD	Occupational exposure to OPs (men)	OR 1.07	0.46	1.70
Hancock (2008)	PD	Ever use of OPs at work or home	OR 1.89	1.11	3.25



17. The pooled Odds Ratio for the three studies was 1.30, 95% CI 0.48-3.53. As in the individual studies, the OR value itself was above 1, but the Confidence Intervals did not suggest significance. The analysis undertaken was a fixed effects analysis, and the I^2 value for heterogeneity between the studies is 0%, p=0.876.

18. Manthripragada et al 2010 is the fifth case-control study to consider. This study was also discussed in the Polymorphisms section (Annex 10, Table 7.B.2, Manthripragada 2010), where the nature of the study group and the effect of the PON1 polymorphism are described in greater detail. The subjects were drawn from the same study population as in Gatto et al 2009, and the study was also conducted as part of the UCLA Parkinson's Environment and Genes Study. In this instance,

351 incident cases of PD were included. Diagnosis and confirmation of diagnosis were performed in the same way as described in paragraph 13. This study further included genotyping of subjects and an analysis for a possible association between PD, exposure to the OPs diazinon, chlorpyrifos and parathion, and the influence of a variant of the PON1 gene at position 55. Exposure was estimated by GIS modelling, as in the Gatto study, with the inherent limitations of this approach regarding specific individual exposure. Results for residential exposure to diazinon, OR=1.55 (1.05-2.30), and chlorpyrifos, OR=1.56 (1.02-2.40), were suggestive of a possible association between exposure and PD, while exposure to parathion did not appear to be significantly associated, OR=0.98 (0.65-1.48) (Annex 9, Table 7.B.2, Manthripragada 2010,"Residential exposure to specific pesticides and association with PD").

Cross-Sectional Study of Brazilian Tobacco Workers

19. Salvi et al 2003 looked at 37 Brazilian workers involved in family agriculture of tobacco and their score for parkinsonism on the Extrapyramidal Symptom Rating Scale (ESRS). The ESRS includes responses to a questionnaire and the findings of a physical examination, but does not give a diagnosis of PD. Subjects were questioned while exposed to OPs, and again after 3 months of no exposure to OPs. Twelve of the subjects were lost to follow-up. A reduction of extrapyramidal symptoms was seen, but 9 subjects out of 25 still had clinically significant symptoms (Annex 9, Table 7.B.3, Salvi 2003, "Scores on the Extrapyramidal Symptom Rating Scale in tobacco workers during exposure to OPs and after 3 months of no exposure to OPs"). The p value for the questionnaire, $p<0.001$, suggested significance of the results, although the physical examination value is borderline ($p=0.056$). This study had a small number of subjects, with a considerable number lost to follow-up after 3 months, and did not include any unexposed subjects for comparison. Furthermore, the study authors reported frequent co-use by subjects of at least one other chemical compound besides OPs, so the results were unlikely to relate exclusively to OPs.

CONCLUSIONS ON CHRONIC LOW-LEVEL EXPOSURE

20. Overall, the evidence for an association between chronic low-level exposure to OPs and PD was limited. Five of the studies analyse a potential association between PD and exposure to OPs as a chemical group. Of these, Engel et al 2001 is a cohort study, analysing results for over 200 men from a well-established cohort recruited in the 1970s, before subjects were likely to exhibit signs of parkinsonism. The study did not find an increased prevalence of parkinsonism associated with exposure to OPs. Elbaz et al 2009, Firestone et al 2005 and Hancock et al 2008 are the three case-control studies compared in our small meta-analysis in paragraph 15, and, although all three report OR values above 1, only the Hancock results have a CI which could suggest significance, OR= 1.89, 95% CI 1.11-3.25. The Salvi et al 2003 cross-sectional study reported positive results on the Extrapyramidal Symptom Rating Scale, but was limited by small study size and the lack of a comparison group.

21. Data relating to individual OP pesticides was limited. The two substances considered by several studies were chlorpyrifos and diazinon. Four studies reported on effects of chlorpyrifos exposure. Albers et al 2004b and Kamel et al 2007a reported no association between exposure to chlorpyrifos and PD; Albers et al 2004b was a well-designed study incorporating measures of exposure, while Kamel et al 2007a considered thousands of subjects enrolled in the US Agricultural Health Study. Gatto et al 2009 and Manthripragada et al 2010 were case-control studies which used the same study population; they reported positive associations between exposure to chlorpyrifos and PD, but their investigation concerned use of well water, which was likely to include a range of contaminants and therefore co-exposure of subjects to other substances, and GIS-based information on environmental exposure, which did not measure individual exposure.

22. There were four studies which reported on exposure to diazinon. The large Kamel et al 2007a cohort study again found no association, as did Engel et al 2001, reporting on subjects taken from a long-established cohort of men in Washington State. The Gatto et al 2009 and Manthripragada et al 2010 studies reported positive associations, as for chlorpyrifos above, but the same limitations apply.

23. The findings do not present a consistent picture for the possibility of an association between exposure to OPs in several settings and the development of PD or parkinsonism. The amount of available evidence was limited, and there was insufficient data at present to establish causation for the chemical class of OPs, or for specific individual OP pesticides.

Table 6. PARKINSON'S DISEASE / PARKINSONISM STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
A. Acute Exposure with Chronic Effects									
6.A.1. Cohort studies - none									
6.A.2. Case-control studies									
Das	2011	India	345 cases of idiopathic Parkinson's Disease (215 males, 130 females, mean age 62±2 years), attending the Neurology Department of Burdwan Medical College and Hospital between January 2003-January 2008	370 controls (220 males, 150 females, mean age 62±3 years), age-matched±3 years and sex-matched, selected from persons accompanying the cases, patients attending general neurology or medicine department of the hospital, or relatives of cases. Controls had neither significant neurological or medical illness	Interview conducted by neurologist; diagnosis of PD was clinically confirmed by a neurologist on the basis of the UK PD Society Brain Bank criteria, with the presence of at least 3 cardinal signs out of: rest tremor, rigidity, bradykinesia, impaired postural reflexes	Parkinson's Disease	Self-reported exposure to environmental risk factors, including source of drinking water and contact with insecticides or pesticides (not specific to OPs); history of acute poisoning taken, which was poisoning by OPs	Multiple factors included in study, each factor analysed separately; history of addiction factors included, which considered smoking habits, alcohol intake, and coffee and tea intake	Logistic regression analysis considering association between PD and multiple factors; estimated coefficient values in logistic regression equation reported, *p* values, and 95% CI for difference of proportion
6.A.3. Cross-sectional studies - none									
6.A.4. Case series studies - none									
6.A.5. Case reports - none									
B. Chronic Low-Level Exposure									
6.B.1. Cohort Studies									
Albers	2004b	USA	All Dow employees engaged in manufacture of chlorpyrifos (aged	Dow employees engaged in manufacture of Saran (plastic film)	Evaluation by board-certified neurologist, and self-report on neurological	Parkinsonism and evaluation of extra-pyramidal	Cumulative exposure to chlorpyrifos estimated from air monitoring data from	Chlorpyrifos exposed and referents were matched for age,	Comparison of results for the chlorpyrifos group and the referent

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			18-65y) who were employed on 1 Sept 1999. Of 66 potentially eligible, 53 took part (80%). All these subjects took part in 1 year follow up	with no known exposure to neurotoxicants. Out of 74 workers asked, 60 (81%) participated, with 58 also participating at 1 year follow-up	questionnaire. For extrapyramidal motor system rating, 3 cardinal motor signs of PD used: abnormal alternate motion rate or bradykinesia, resting tremor of any extremity, and abnormal gait, tone or posture or postural instability. Rating included major motor components of the Unified Parkinson's Disease Rating Scale (UPDRS)	signs; the study did not seek to diagnose Parkinson's Disease	initial employment to baseline and from baseline to 1 year follow-up. Urinary excretion of 3,5,6-trichloro-2-pyridinol (TCP) measured between baseline and 1-year follow-up, corrected for urinary creatine, average of 4 nightly collections. Plasma butyryl-cholinesterase (BuChE) and red blood cell cholinesterase (AChE) measured	height, weight, BMI, reading scale, smoking pack year and anxiety	group at baseline and 1 year follow-up undertaken by *t* test for difference in means, Fisher exact test or chi-squared test for associations; *P* value reported
Engel	2001	USA	238 exposed subjects from an original cohort of 1300 subjects, enrolled 1972-76, for the study of men occupationally exposed to pesticides in Washington State; subjects in the current study were asked to participate in summer 1997. Occupations included were: orchardists, professional pesticide applicators, pesticide formulation plant workers, other farm or agricultural workers	72 non-exposed subjects identified from a City Directory, frequency matched with exposed subjects by age, race, and degree of occupational physical activity; occupations included construction workers, lorry drivers, road workers, roofers, salesmen	A nurse trained in neurological examinations administered a 20 minute uniform neurological examination to all subjects, using the Unified Parkinson's Disease Rating Scale (UPDRS)	Parkinsonism	Self-reported exposure on questionnaire to specific insecticides, including OPs, and to specific OPs	Adjustments made for age and pack-years of smoking	Generalised linear model with binomial distribution used to estimate prevalence ratios (PRs) with 95% CIs
Kamel	2007a	USA	83 participants who reported physician-	79,557 cohort members who did	Cases self-reported physician-diagnosed	Parkinson's Disease	Self-reported duration (years) and	Adjustments made for: age at	Logistic regression to evaluate the relation

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			diagnosed PD at enrolment, were defined as "prevalent cases"; 78 participants who reported physician-diagnosed PD at follow-up were defined as "incident cases"	not report PD at enrolment were the comparison population for the 83 prevalent cases; 55,931 cohort members who did not report PD at follow-up were the comparison population for the 78 incident cases	PD at enrolment or follow-up		frequency (days per year) of use of any pesticide, and ever use of 50 commonly used pesticides, including specific OPs	enrolment, using a 4-level categorical variable with persons aged 51-60 years as the referent group; State (Iowa or North Carolina); type of participant (applicator or spouse) in models that included both. Race and education also considered as possible confounders	of either prevalent PD or incident PD to general pesticide variables; two-stage hierarchical logistic regression used when evaluating multiple specific pesticides; ORs and 95% CIs reported
6.B.2. Case-control studies									
Elbaz	2009	France	224 cases from the Mutualité Sociale Agricole in France who had applied for healthcare for PD from February 1998 to August 1999	557 controls who were affiliates of the same health insurance, free of PD, and who had requested reimbursement for health-related expenses from February 1998 to February 2000; maximum of 3 controls matched to each case, on age ±2 years, sex, and affiliation office	Examination by movement disorders neurologist, or, if not possible, patients' neurologists contacted; parkinsonism defines as presence of 2 or more cardinal signs from rest tremor, bradykinesia, rigidity, impaired postural reflexes; PD defined by parkinsonism, after exclusion of prominent or early signs of more extensive nervous system involvement and drug-induced parkinsonism	Parkinson's Disease	Self-reported exposure on questionnaire and in interview	Analyses were stratified by sex, median age at onset/ index age, and median disease duration; all analyses were adjusted for cigarette smoking (pack-years) and for the results of the Mini Mental State Examination (MMSE), a test used to assess cognitive impairment	Complete-case analyses conducted using conditional logistic regression; multiple imputation analysis also performed, with missing values replaced by plausible values based on observed data for that variable, using logistic regression model; ORs and 95% CIs reported, and *p* value for trend in exposure to categories of pesticides (insecticides/ fungicides/ herbicides)
Firestone	2005	USA	250 newly diagnosed, idiopathic PD case	388 randomly selected subjects from those enrolled	PD diagnosis confirmed by medical chart review by trained	Parkinson's Disease	Self-reported exposure using checklist of common	Adjustments for age, sex, smoking	Unconditional logistic regression models used, reporting

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			patients identified between 1992 and 2002 at the Group Health Cooperative in western Washington State or the University of Washington	at the GHC (Group Health Cooperative), who had no history of PD or other progressive neurological disorder. Controls matched to cases by age in 10-year categories, sex, GHC location and original year of GHC enrolment; they were *not* matched by historical exposures, occupation, or place of residence	neurologist; diagnosis of PD required at least 2 of the 4 cardinal signs of PD (bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment), one of which had to be bradykinesia or resting tremor; exclusion criteria to exclude other possible causes of parkinsonism applied; combination of measures used (health provider referrals, computerised databases containing diagnostic coding, pharmacy information) to ensure complete ascertainment		chemical agents/ commercial brand name products; occupational exposure in men based on having personally worked on machines that sprayed chemicals, applied pesticide sprays or powders by hand, or worked in area recently sprayed with chemicals; home-based exposure defined as personal use of a product in and around the home		adjusted ORs and 95% CIs
Gatto	2009	USA	Study conducted as part of the UCLA (University of California-Los Angeles) Parkinson's Environment and Genes Study; 368 cases, recruited by local neurologists, through PD support groups and local newspapers, from largely agricultural population in California; subjects recruited between 2001-2007, resided in Fresno, Tulare, or Kern Counties, and	341 population controls, recruited from randomly selected tax assessor residential units in each of the three counties, and through letters of invitation to random selection of residential living units. Controls had to be at least 35, currently residing in one of the 3 counties, and to have lived in California for at least 5 years before the screening	Diagnoses of PD confirmed by UCLA movement disorder specialist. Diagnosis of clinically probable PD confirmed if patients had at least 2 of the symptoms:- resting tremor, bradykinesia, or cogwheel rigidity, and no suggestion of parkinsonian syndrome due to other causes such as trauma or brain tumour, or any other atypical features such as cerebellar or pyramidal signs	Parkinson's Disease	Geographic information system (GIS)-based model to estimate potential well-water contamination from agricultural pesticides; addresses in Fresno, Kern and Tulare counties between 1974-1999 used. Ambient pesticide application rates from agricultural uses within 500 metres of each subject's home were estimated by the validated GIS-based system, which	Age, sex, education, race/ethnicity, family history of PD, smoking	Multivariable unconditional logistic regression, reporting ORs and 95% CIs

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			had lived in California for at least 5 years before diagnosis or interview				combined California Pesticide Use Reports (PURs) data and land-use maps		
Hancock	2008	USA	319 cases from 308 families, recruited between 2000 – 2006 by the Udall PD Research Center at Duke University Medical Center	296 relative and other controls; of these, 252 were relative controls from the 308 families with at least one case of Parkinson's Disease, so referred cases were largely matched to family controls; the remaining 44 controls were either spouses or other unrelated individuals	medical history questionnaire; clinical examination by a board-certified neurologist. All individuals were given a standard in-person clinical examination, using the full Unified PD Rating Scale (UPDRS). Subjects considered to have PD demonstrated at least two cardinal signs of PD (resting tremor, rigidity, and/or bradykinesia), an asymmetry of symptom onset, and no atypical signs. A board-certified neurologist, physician assistant, or registered nurse also examined unaffected individuals who had no signs of PD, and unclear individuals, who had only one cardinal sign	Parkinson's Disease	Self-reported application of pesticides at work, at home, or in the garden; individuals were asked to list the names of any pesticides they remembered using	Models were adjusted for age-at-examination, sex, cigarette smoking, caffeine consumption. Families were also stratified by self-reported race/ethnicity; only white families provided sufficient statistical power, so analyses performed on this subset only	Population-averaged generalised estimating equations (GEE), as implemented by SAS version 8e, were used to model associations between pesticide exposure and PD. Two types of GEE models constructed, one testing for the trend of effects, and the second for the effect of each exposure category (ever versus never, or high, moderate and low versus never). ORs and 95% CIs reported
Manthri-pragada	2010	USA	Study conducted as part of the UCLA Parkinson's Environment and Genes Study; 351 incident cases, from the same study population as in the Gatto 2009 study above, recruited by local	363 population controls , recruited from randomly selected tax assessor residential units in each of the three counties,or identified through a random sample of Medicare enrolees	Eligible cases were examined by a UCLA movement disorder specialist and were confirmed as having clinically "probable" or "possible" Parkinson's Disease; DNA sample taken for genotyping, using whole blood or buccal	Parkinson's Disease	Residential pesticide exposure estimated for each participant using their residential history and the GIS-based system described for the Gatto 2009 study above; occupational pesticide exposure	All effect estimates adjusted for sex, smoking status (ever/never), age, education, county (Fresno, Tulare or Kern), race. In some analyses adjustment made for occupational pesticide exposure	Genotyping performed for PON1-55; Hardy-Weinberg equilibrium for PON1-55 in controls assessed using χ^2 test; genotype frequencies compared in cases and controls;

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			neurologists, through PD support groups and local newspapers, from a largely agricultural population in California; subjects were recruited between 2001 to 1 January 2008, resided in Fresno, Tulare, or Kern Counties, and had lived in California for at least 5 years before diagnosis or interview		cell samples		estimates based on lifetime history of occupational titles, and self-reported agricultural pesticide application; specific pesticides examined for association with PD were the OPs diazinon, chlorpyrifos and parathion	(not exposed, possibly, likely exposed), and for exposure to all other pesticides. Also stratification by sex and age at diagnosis (≤60, >60 years) in some analyses	recessive inheritance model used. Unconditional logistic regression used to calculate ORs and 95% CIs for main effects of genes and specific OP pesticides
6.B.3. Cross-sectional studies									
Salvi	2003	Brazil	37 workers involved in family agriculture of tobacco in December 2001, when they had been working with OPs for 3 months; second evaluation in March 2002 conducted in 25 workers from the original sample who returned for the follow-up, when they had been off OP exposure for 3 months	No unexposed group is included	The Extrapyramidal Symptom Rating Scale (ESRS) was used: includes a questionnaire for complaints and a physical examination concerning parkinsonian symptoms	Parkinsonism, parkinsonian symptoms	plasma AchE levels	time and type of OP exposure, use of protective clothing, activities with OPs, history of acute toxicity, alcohol and tobacco use were noted as characteristics of the 25 subjects who completed both evaluations; possible bias because affected subjects might have enrolled more readily in the study, and there is no unexposed comparison group	Comparisons between the two evaluations performed using Wilcoxon test for asymmetric variables such as the Scale scores; statistical significance defined as $p<0.05$
6.B.4. Case series studies - none									

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
6.B.5. Case reports - none									

Table 7. PARKINSON'S DISEASE / PARKINSONISM RESULTS AND CONCLUSIONS

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td colspan="5">A. Acute Exposure with Chronic Effects</td></tr>
<tr><td colspan="5">7.A.2. Case-control studies</td></tr>
<tr><td>Das</td><td>2011</td><td>STUDY OF THE ROLE OF FAMILIAL, ENVIRONMENTAL AND OCCUPATIONAL FACTORS IN THE DEVELOPMENT OF PD IN A STUDY IN INDIA

EXPOSURE Self-reported acute OP poisoning

HEALTH EFFECTS The only OP-specific information in the study relates to the effects of acute poisoning; the tables below indicate the number of cases and controls affected, and the significance of the findings:

Numbers of cases and controls with acute OP poisoning
<table>
<tr><th>Characteristic</th><th>Cases (n=345)</th><th>Controls (n=370)</th></tr>
<tr><td>History of acute poisoning</td><td></td><td></td></tr>
<tr><td>Organophosphate</td><td>8 (2.31%)</td><td>2(0.54%)</td></tr>
<tr><td>Others</td><td>0</td><td>0</td></tr>
</table>
p value and 95% CI for the difference of proportion for acute OP poisoning
<table>
<tr><th>Factor</th><th>p value</th><th>95% CI for the difference of proportion</th></tr>
<tr><td>Acute OP poisoning</td><td>0.046</td><td>(0.002-0.038)</td></tr>
</table></td><td><u>Strengths</u>
Relatively large number of matched cases and controls in study

Clinical diagnosis by neurologist, cases taken from specialist neurological referral centre

<u>Limitations</u>
Very small number of OP-poisoned cases and controls suffering from PD

History of OP poisoning is self-reported</td><td>Results of the study suggest possible association between acute OP poisoning and subsequent development of PD, but the number of exposed subjects is very small</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>Estimated coefficient value in logistic regression equation
<table><tr><th>Factor</th><th>p value</th><th>Estimated coefficient value</th></tr><tr><td>Acute OP poisoning</td><td>0.046</td><td>1.57479</td></tr></table>Thus, the p values suggest marginal significance for the association between a history of acute OP poisoning and the development of PD; 8 cases and 2 controls had developed the condition</td><td></td><td></td></tr>
<tr><td colspan="5">B. Chronic Low-Level Exposure</td></tr>
<tr><td colspan="5">7.B.1. Cohort studies</td></tr>
<tr><td>Albers</td><td>2004b</td><td>STUDY OF EFFECTS OF EXPOSURE TO CHLORPYRIFOS IN CHLORPYRIFOS PRODUCTION WORKERS

EXPOSURE Urinary excretion of OP metabolites measured: excretion of 3,5,6 trichloro-2-pyridinol (TCP) significantly higher in chlorpyrifos-exposed subjects (192.2 µg/g creatinine vs 6.2 µg/g creatinine in referents) (P<0.0001). Plasma butyrylcholinesterase (BuChE) activity significantly lower in chlorpyrifos-exposed subjects (7155 vs 8183 mu/ml; P<0.01). Ratio of lowest BuChE during follow-up period to baseline was significantly lower in chlorpyrifos exposed subjects. Daily excretion of TCP equated to systemic daily doses of 576-627 µg/day. There was no effect on acetylcholinesterase, as AChE levels were similar for both groups at baseline and 1-year follow-up examinations.

HEALTH EFFECTS Aim of neurological examinations was to evaluate extrapyramidal signs and to assess the presence of parkinsonism, for the purpose of categorising subjects as having possible, or probable, extrapyramidal dysfunction. Diagnosis of PD not attempted.

The table below shows the results for comparison of clinical examination results relevant to the central nervous system evaluation for chlorpyrifos and referent subjects at baseline (Chlorpyrifos subjects n=53, referents n=60) and at the 1 year follow-up (Chlorpyrifos subjects n=53, referents n=58), and the neurological ratings for subjects at both evaluations:</td><td><u>Strengths</u>
Biomonitoring measures are available for subjects and referents in the study

Prospective design and accurate estimation of exposure to chlorpyrifos

<u>Limitations</u>
Small numbers of study subjects</td><td>No clinically apparent extrapyramidal signs associated with Parkinsonism were observed in this study</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
Clinical examination results in chlorpyrifos production workers
<table>
<tr><th>Measure</th><th>Baseline</th><th>No.</th><th>P-value*</th><th>Second</th><th>No.</th><th>P-value*</th></tr>
<tr><td>Postural tremor</td><td>CPF</td><td>2</td><td></td><td>CPF</td><td>2</td><td></td></tr>
<tr><td></td><td>Referent</td><td>1</td><td>0.60</td><td>Referent</td><td>1</td><td>0.61</td></tr>
<tr><td>Resting tremor</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
<tr><td>Abnormal alternate motion rate</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
<tr><td>Bradykinesia</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
<tr><td>Increased tone</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
<tr><td>Abnormal station or gait</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
<tr><td>Neurological rating</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Extrapyramidal motor system:
any extrapyramidal abnormality</td><td>CPF</td><td>0</td><td></td><td>CPF</td><td>0</td><td></td></tr>
<tr><td></td><td>Referent</td><td>0</td><td></td><td>Referent</td><td>0</td><td></td></tr>
</table>
Notes: * Fisher’s exact two-tailed test
CPF= chlorpyrifos

Apart from postural tremor, no subject showed any extrapyramidal signs at either examination. One subject showing an enhanced postural tremor on both examinations was taking a medication associated with tremor. Overall the P value for postural tremor does not indicate significance
</td><td></td><td></td></tr>
<tr><td>Engel</td><td>2001</td><td>STUDY OF RISK OF PARKINSONISM IN MEN OCCUPATIONALLY EXPOSED TO PESTICIDES

EXPOSURE Self-reported exposure to OPs and to azinphos methyl, diazinon, methyl parathion. Measure of years of exposure was grouped into tertiles, and also the number of acre-years (number of years x number of acres of crops to which substance was applied)

HEALTH EFFECTS
Parkinsonism prevalence ratios (PRs, ever or never) by exposure related to pesticides</td><td><u>Strengths</u>
Subjects belonged to a cohort established in the 1970s, before most of them were likely to show signs of parkinsonism, which minimises selection bias</td><td>The results do not suggest an increased prevalence of parkinsonism associated with exposure to OPs, or with three specific OPs studied</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><th rowspan="2">Exposure</th><th colspan="2">Cases (n(%))</th><th colspan="2">PR (95% CI) for parkinsonism</th></tr>
<tr><th>Exposed</th><th>Non-exposed</th><th>Unadjusted</th><th>Adjusted*</th></tr>
<tr><td>Any OPs</td><td>48(21.0)</td><td>17(21.0)</td><td>1.0(0.6-1.6)</td><td>0.9(0.6-1.4)</td></tr>
<tr><td>Azinphos methyl</td><td>24(17.5)</td><td>24(21.1)</td><td>0.8(0.5-1.4)</td><td>0.8(0.5-1.3)</td></tr>
<tr><td>Diazinon</td><td>41(20.5)</td><td>24(22.2)</td><td>0.9(0.6-1.4)</td><td>0.8(0.5-1.3)</td></tr>
<tr><td>Methyl parathion</td><td>28(18.1)</td><td>36(24.0)</td><td>0.7(0.5-1.2)</td><td>0.7(0.5-1.1)</td></tr>
</table>
Note * adjusted for age and pack-years of smoking

Parkinsonism prevalence ratios (PRs) by tertiles of years of exposure related to pesticides
<table>
<tr><th rowspan="2">Factor</th><th colspan="3">PR (95% CI) of Parkinsonism*
Unadjusted</th><th colspan="2">Adjusted**</th></tr>
<tr><th>Tertile 1</th><th>Tertile 2</th><th>Tertile 3</th><th>Tertile 2</th><th>Tertile 3</th></tr>
<tr><td>Any OP</td><td>1.0</td><td>1.2(0.7-2.2)</td><td>0.9(0.4-1.7)</td><td>1.3(0.7-2.3)</td><td>0.8(0.4-1.5)</td></tr>
<tr><td>Azinphos methyl</td><td>1.0</td><td>0.4(0.2-1.1)</td><td>0.4(0.2-1.1)</td><td>0.5(0.2-1.0)</td><td>0.6(0.3-1.4)</td></tr>
<tr><td>Diazinon</td><td>1.0</td><td>1.0(0.5-1.9)</td><td>1.0(0.5-2.0)</td><td>1.0(0.5-2.0)</td><td>1.1(0.6-2.2)</td></tr>
<tr><td>Methyl parathion</td><td>1.0</td><td>1.0(0.4-2.3)</td><td>1.2(0.5-2.7)</td><td>1.0(0.4-2.4)</td><td>1.2(0.5-3.0)</td></tr>
</table>
Notes * restricted to subjects with the particular exposure
** adjusted for age and pack-years of smoking

Parkinsonism prevalence ratios (PRs) by tertiles of acre-years of exposure related to pesticides
<table>
<tr><th rowspan="2">Factor</th><th colspan="3">PR (95% CI) of Parkinsonism*
Unadjusted</th><th colspan="2">Adjusted**</th></tr>
<tr><th>Tertile 1</th><th>Tertile 2</th><th>Tertile 3</th><th>Tertile 2</th><th>Tertile 3</th></tr>
<tr><td>Any OP</td><td>1.0</td><td>0.7(0.4-1.3)</td><td>0.9(0.5-1.7)</td><td>0.7(0.4-1.2)</td><td>0.9(0.5-1.6)</td></tr>
<tr><td>Azinphos methyl</td><td>1.0</td><td>1.0(0.4-2.4)</td><td>0.7(0.3-1.9)</td><td>1.2(0.5-2.8)</td><td>0.9(0.4-2.2)</td></tr>
<tr><td>Diazinon</td><td>1.0</td><td>0.7(0.3-1.6)</td><td>1.1(0.5-2.2)</td><td>0.8(0.4-1.6)</td><td>1.2(0.6-2.4)</td></tr>
<tr><td>Methyl parathion</td><td>1.0</td><td>1.3(0.5-3.1)</td><td>1.1(0.5-2.9)</td><td>1.1(0.5-2.7)</td><td>1.3(0.5-3.0)</td></tr>
</table>
Notes * restricted to subjects with the particular exposure
** adjusted for age and pack-years of smoking

The results above do not suggest association between exposure to any OPs, or to azinphos methyl, diazinon, or methyl parathion, and the risk of Parkinsonism
</td>
<td>
<u>Limitations</u>
Cases of parkinsonism in the study presented with only slight or mild signs, so difficulty of assessing the signs might have resulted in some misclassification

Incomplete follow-up of the original cohort (attempt to contact only 56.9% of original cohort , and only 67% of those subjects agreed to participate)
</td>
<td></td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td>Kamel</td><td>2007a</td><td>STUDY OF PESTICIDE EXPOSURE AND PARKINSON'S DISEASE IN AGRICULTURAL HEALTH STUDY COHORT

EXPOSURE Self-reported duration and frequency of use of 10 specific OPs
HEALTH EFFECTS The study analysed participants enrolled in the Agricultural Health Study cohort with reported physician-diagnosed PD at enrollment ("prevalent" cases), and participants who developed PD by follow-up ("incident" cases). The results for 10 specific OPs are given in the table below:

Prevalent and Incident PD in Agricultural Health Study participants who used specific OP pesticides

<table>
<tr><th rowspan="2">Chemical</th><th colspan="4">Prevalent PD</th><th rowspan="2">OR(95%CI)</th><th colspan="4">Incident PD</th><th rowspan="2">OR(95%CI)</th></tr>
<tr><th colspan="2">Cases</th><th colspan="2">Controls</th><th colspan="2">Cases</th><th colspan="2">Controls</th></tr>
<tr><th></th><th>No.</th><th>%</th><th>No.</th><th>%</th><th></th><th>No.</th><th>%</th><th>No.</th><th>%</th><th></th></tr>
<tr><td>Glyphosate</td><td>45</td><td>55</td><td>46,687</td><td>60</td><td>1.0(0.6-1.7)</td><td>49</td><td>67</td><td>32,686</td><td>60</td><td>1.1(0.6-2.0)</td></tr>
<tr><td>Dichlorvos</td><td>4</td><td>5</td><td>5,285</td><td>7</td><td>0.8(0.4-1.9)</td><td>8</td><td>11</td><td>3,959</td><td>8</td><td>0.7(0.3-1.4)</td></tr>
<tr><td>Chlorpyrifos</td><td>25</td><td>30</td><td>21,380</td><td>28</td><td>1.2(0.7-2.1)</td><td>24</td><td>33</td><td>14,570</td><td>27</td><td>0.9(0.5-1.6)</td></tr>
<tr><td>Coumaphos</td><td>4</td><td>5</td><td>4,185</td><td>6</td><td>0.8(0.3-1.9)</td><td>6</td><td>9</td><td>3,040</td><td>6</td><td>0.8(0.4-1.9)</td></tr>
<tr><td>Diazinon</td><td>17</td><td>24</td><td>17,519</td><td>23</td><td>1.0(0.5-1.8)</td><td>21</td><td>30</td><td>12,276</td><td>24</td><td>0.9(0.5-1.7)</td></tr>
<tr><td>Fonofos</td><td>11</td><td>14</td><td>10,461</td><td>14</td><td>0.9(0.3-1.7)</td><td>12</td><td>17</td><td>7,121</td><td>14</td><td>1.0(0.5-1.8)</td></tr>
<tr><td>Malathion</td><td>41</td><td>55</td><td>38,292</td><td>50</td><td>1.1(0.6-2.0)</td><td>49</td><td>67</td><td>26,577</td><td>51</td><td>1.2(0.6-2.1)</td></tr>
<tr><td>Parathion</td><td>11</td><td>15</td><td>7,338</td><td>10</td><td>1.3(0.6-2.7)</td><td>14</td><td>20</td><td>4,962</td><td>10</td><td>1.1(0.6-2.2)</td></tr>
<tr><td>Phorate</td><td>20</td><td>28</td><td>15,528</td><td>21</td><td>1.1(0.6-2.0)</td><td>26</td><td>37</td><td>10,402</td><td>20</td><td>1.4(0.8-2.5)</td></tr>
<tr><td>Terbufos</td><td>22</td><td>28</td><td>18,805</td><td>25</td><td>0.9(0.5-1.7)</td><td>23</td><td>33</td><td>12,693</td><td>25</td><td>1.1(0.6-2.0)</td></tr>
</table>

The results for these specific OPs do not suggest a significant association between exposure to the chemical and the development of PD, in prevalent or incident cases. The study did find a positive association with overall pesticide use and incident PD, for the highest quartile of cumulative days of pesticide use against the lowest</td><td><u>Strengths</u>
It was possible to analyse the effects of a number of specific OPs in this study because of the large size of the cohort

Study was able to distinguish between prevalent and incident cases

<u>Limitations</u>
Individuals were exposed to multiple pesticides

Only 68% of the original cohort were interviewed at follow-up

Self-report of physician-diagnosed PD, so disease misclassification possible</td><td>The results do not suggest an association between exposure to 10 specific OPs and prevalent or incident PD, although the study did find a positive association between overall pesticide use and incident PD in subjects with the highest exposure</td></tr>
<tr><td colspan="5">7.B.2. Case-control studies</td></tr>
<tr><td>Elbaz</td><td>2009</td><td>FRENCH STUDY OF WORKERS IN AGRICULTURE WITH PROFESSIONAL EXPOSURE TO SPECIFIC PESTICIDE FAMILIES

EXPOSURE Self-reported occupational history including information on pesticides used, frequency of use (days/year), duration (hours/year), spraying method and start/end years

HEALTH EFFECTS
Results for pesticide and insecticide exposure</td><td><u>Strengths</u>
Three types of exposure are considered and compared in the study: exposure to pesticides, to the broad category of</td><td>Although the study does not find a significant association between PD and specific exposure to OPs, a significant</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>The study included 224 cases and 557 matched controls.

The overall result for exposure through professional pesticide use was: OR=1.8, 95% CI 1.1-3.1, with a dose-effect relation found for the number of years of use, p=0.01. For the category of insecticides, the association of PD with professional use was OR=2.2, 95% CI 1.1-4.3, p=0.03 for men, and OR=1.4, 95% CI 0.5-3.8, p=0.49 for women
Results for exposure to OPs
The table below gives the results for men, analysed by two different methods, and exposure to OP insecticides:

<table>
<tr><th>Exposure</th><th colspan="2">Complete-Case Analysis</th><th colspan="2">Multiple Imputation</th></tr>
<tr><th></th><th>Men, OR (95% CI)</th><th>Men, AO>65yr, OR (95% CI)</th><th>Men, OR (95% CI)</th><th>Men, AO>65yr, OR (95% CI)</th></tr>
<tr><td>Organophosphorus insecticides</td><td>1.8(0.9-3.7)</td><td>2.9(0.9-9.1)</td><td>1.3(0.7-2.3)</td><td>1.6(0.7-3.7)</td></tr>
</table>

Notes: AO = age at onset;
in the Complete-Case analysis, the method involves deleting observations with missing information from the analysis; in Multiple Imputation, missing values are replaced in the analysis with plausible values based on observed data

Thus, although the results for men's exposure to insecticides appear to be significant, the CIs for specific exposure to OPs do not suggest significance</td>
<td>insecticides, and to the specific chemical family of OPs

Relatively large number of cases and controls

Detailed information gathered regarding exposure, including visit of interviewers to farms where subjects worked, checking old pesticide containers and reviewing bills and farming calendars as sources of information about substances used

<u>Limitations</u>
Despite detailed questions, exposure remains self-reported</td>
<td>association is found with the broader categories of insecticides and pesticides</td>
</tr>
<tr>
<td>Firestone</td>
<td>2005</td>
<td>POPULATION-BASED STUDY OF PD IN WESTERN WASHINGTON STATE, REPORTING ON 250 INCIDENT PD CASE PATIENTS AND CONTROLS

EXPOSURE Self-reported exposure in interview of occupational and home-based pesticide use, drinking water source and residential and smoking history. Estimates derived from first and last year of use and frequency of exposure (number of exposed days per year). Exposures during 5 years before interview date discounted to avoid counting exposures after disease onset; controls' exposure histories discounted similarly. Cumulative exposures calculated as product of duration and frequency of use.

HEALTH EFFECTS The table below shows the results for occupational exposures and risk of Parkinson's Disease in men:

PD in cases and controls by occupational title and exposure to specific OPs</td>
<td><u>Strengths</u>
PD diagnosis was confirmed by trained neurologists, and a combination of measures was performed to ensure complete ascertainment

Relatively large number of cases and matched controls</td>
<td>The study does not find significant associations between exposure to OPs occupationally or in the home and PD. The authors comment that PD is increasingly seen not as a single disorder but as a result of the interaction of</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><th>Variable</th><th>Cases (n=156)</th><th>Controls (n=241)</th><th>Odds Ratio (95% CI)</th></tr>
<tr><td>Occupational title</td><td></td><td></td><td></td></tr>
<tr><td>Pesticide worker</td><td>7</td><td>6</td><td>2.07 (0.67-6.38)</td></tr>
<tr><td>Crop farmer</td><td>19</td><td>20</td><td>1.65 (0.84-3.27)</td></tr>
<tr><td>Animal and crop farmer</td><td>21</td><td>33</td><td>1.10 (0.60-2.00)</td></tr>
<tr><td>Dairy farmer</td><td>16</td><td>30</td><td>0.88 (0.46-1.70)</td></tr>
<tr><td>Organophosphates</td><td>11</td><td>14</td><td>1.07 (0.46-2.49)</td></tr>
<tr><td>Parathion</td><td>5</td><td>1</td><td>8.08 (0.92-70.85)</td></tr>
<tr><td>Diazinon</td><td>6</td><td>9</td><td>1.04 (0.35-2.72)</td></tr>
<tr><td>Malathion</td><td>8</td><td>10</td><td>1.01 (0.37-2.72)</td></tr>
</table>

The 95% CIs of the above ORs do not suggest significance. However, the authors comment that their study examines a predominantly urban population, not particularly enriched for agricultural exposures. The ORs themselves appear to show a gradient of risk for occupational titles that parallels the predicted level of exposure (pesticide worker>crop farmer>combined animal and crop farmer>dairy farmer).

The table below shows home-based exposures and risk of Parkinson's Disease in all subjects:

PD in cases and controls and home-based exposures

<table>
<tr><th>Variable</th><th>Cases (n=250)</th><th>Controls (n=241)</th><th>Odds Ratio (95% CI)</th></tr>
<tr><td>Organophosphates</td><td>113</td><td>193</td><td>0.83 (0.60-1.16)</td></tr>
<tr><td>Well water use</td><td></td><td></td><td></td></tr>
<tr><td>Ever</td><td>105</td><td>162</td><td>1.08 (0.77-1.50)</td></tr>
<tr><td>Lifelong</td><td>28</td><td>25</td><td>1.81 (1.02-3.21)</td></tr>
<tr><td>Residence</td><td></td><td></td><td></td></tr>
<tr><td>Farm at age <25y</td><td>62</td><td>87</td><td>1.21 (0.82-1.79)</td></tr>
<tr><td>Farm at age ≥25y</td><td>4</td><td>10</td><td>0.63 (0.19-2.08)</td></tr>
<tr><td>Agriculture region</td><td>45</td><td>56</td><td>1.31 (0.84-2.03)</td></tr>
</table>

Of all these exposures, only well water use throughout life was associated with significantly increased risk; however, exposure through use of well water would likely be exposure to multiple pesticides, not only OPs. No significant trend was seen either with duration or cumulative exposure to home-based pesticides, and the OR for home-based exposure to OPs in the table above does not suggest significance
</td>
<td><u>Limitations</u>
Numbers of cases and controls occupationally exposed to OPs are small

Occupational title can only give an estimate of exposure, not any direct measure, and well water use as well as farm residence are only surrogates for pesticide exposure

Only a limited amount of information relates specifically to OP exposure

For home-based exposures, it is likely that, even when OPs are specified as a group, there was co-exposure to other compounds</td>
<td>various environmental and genetic risk factors, so the attributable risk from any single factor is likely to be small</td>
</tr>
<tr>
<td>Gatto</td>
<td>2009</td>
<td>POPULATION-BASED CASE-CONTROL STUDY OF WELL-WATER CONSUMPTION AND PARKINSON'S DISEASE IN RURAL CALIFORNIA</td>
<td><u>Strengths</u>
The GIS-based</td>
<td>The authors report evidence</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
EXPOSURE Estimates of well-water pesticide exposure based on combination of pesticide use and application data and self-reports of private wells as drinking water sources at residential address. 26 chemicals were selected for their potential to pollute groundwater or for their interest in PD, and 6 of those pesticides were examined separately because at least 10% of the study population was exposed to them. The OPs included were diazinon, dimethoate and chlorpyrifos.

16.9% of all subjects reported private well water as their drinking water source at some time during the 1974-1999 (inclusive) period. Cases were more likely to have consumed private well water, and to have consumed it on average 4.3 years longer than controls (p=0.02):

<table>
<tr><th>Characteristic</th><th>Cases (n=368)</th><th>Controls (n=341)</th><th>OR (95% CI)</th></tr>
<tr><td>Ever had private well water</td><td>259 (70.4%)</td><td>234 (68.6%)</td><td>1.21 (0.82-1.80)</td></tr>
</table>

However, the 95% CI does not suggest significance.

HEALTH EFFECTS The results for the relative risk of PD and exposure to specific OPs are shown in the tables below:

Relative risk of PD from potential exposure to individual OP pesticides in well water

<table>
<tr><th rowspan="2">Pesticide</th><th colspan="3">Exposure vs. no exposure</th></tr>
<tr><th>Exposure level</th><th>Cases/controls</th><th>OR (95% CI)</th></tr>
<tr><td>Diazinon</td><td>None</td><td>295/300</td><td>1.0 reference</td></tr>
<tr><td></td><td>Any</td><td>73/41</td><td>1.58 (1.03-2.43)</td></tr>
<tr><td>Dimethoate</td><td>None</td><td>295/290</td><td>1.0 reference</td></tr>
<tr><td></td><td>Any</td><td>78/51</td><td>1.41 (0.94-2.11)</td></tr>
<tr><td>Chlorpyrifos</td><td>None</td><td>301/300</td><td>1.0 reference</td></tr>
<tr><td></td><td>Any</td><td>67/41</td><td>1.45 (0.94-2.24)</td></tr>
</table>

Although all three ORs are above 1, only the CI for diazinon suggests marginal significance.

Relative risk of PD from potential inhalation and ingestion of OP pesticides

<table>
<tr><th>Pesticide/exposure</th><th>Cases/controls</th><th>OR (95% CI)</th></tr>
<tr><td>Diazinon unexposed</td><td>165/188</td><td>1.0 (reference)</td></tr>
<tr><td>Ambient pesticide only</td><td>130/112</td><td>1.29 (0.92-1.81)</td></tr>
<tr><td>Ambient and well water</td><td>73/41</td><td>1.75 (1.12-2.76)</td></tr>
<tr><td>Dimethoate unexposed</td><td>150/180</td><td>1.0 (reference)</td></tr>
</table>
</td>
<td>
model to estimate exposure is a semi-quantitative approach using agricultural pesticide application records to derive exposure estimates, so study authors did not have to rely on subjects' recall of their own pesticide use

Relatively large numbers of cases and controls

All PD diagnoses clinically confirmed by a movement disorder specialist

<u>Limitations</u>
Exposures are likely to have been to multiple pesticides, since several were generally co-applied, so exposures would not have been to OPs alone

Exposure measures are only estimates, since well water was not sampled to directly measure pesticide levels

The well water in
</td>
<td>
suggesting that well water presumably contaminated with pesticides may play a role in the aetiology of PD, but it is difficult to see the specific effects of OPs separately from other pesticides or chemicals, since subjects were likely to have been exposed to a mixture of substances in the water
</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><td>Ambient pesticide only</td><td>140/110</td><td>1.57(1.12-2.22)</td></tr>
<tr><td>Ambient and well water</td><td>78/51</td><td>1.72 (1.12-2.65)</td></tr>
<tr><td>Chlorpyrifos unexposed</td><td>186/210</td><td>1.0 reference</td></tr>
<tr><td>Ambient pesticide only</td><td>115/90</td><td>1.42 (1.00-2.01)</td></tr>
<tr><td>Ambient and well water</td><td>67/41</td><td>1.63 (1.04-2.57)</td></tr>
</table>

Note: “ambient” exposure means exposure to the pesticide applied within 500 metres of subject’s home, as estimated by a Geographic Information System-based model

The above results for exposure to all 3 OP pesticides suggest a significant association with the risk of developing PD (except for ambient exposure only to diazinon, 95% CIs all suggest significance). The relative risk associated with a combined exposure to pesticides in the environment and in presumably contaminated well water is greater than that associated with ambient exposure alone in all 3 cases: this may suggest exposures from consumption of potentially contaminated well water may confer some additional, independent risk above ambient exposure.

The table below suggests that contamination of well water by a greater number of OPs, further increases the risk of PD:

Relative risk of PD from potential exposure to increasing numbers of OPs in well water
<table>
<tr><th colspan="4">Relative risk of PD from potential exposure to pesticides in well water</th></tr>
<tr><th>Pesticide family</th><th>Cases/controls</th><th>OR (95% CI)</th><th>p-Trend</th></tr>
<tr><td>OP pesticides (n=10)</td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>272/227</td><td>1.0 (reference)</td><td></td></tr>
<tr><td>1-4</td><td>36/33</td><td>1.03(0.61-1.74)</td><td></td></tr>
<tr><td>≥5</td><td>60/31</td><td>1.71(1.06-2.76)</td><td>0.04</td></tr>
</table>
However, use of well water is likely to involve co-exposure to other pesticides besides OPs, so it is not possible to say that the risk of PD is associated specifically with exposure to OPs
</td>
<td>rural areas may be contaminated with multiple agricultural and industrial agents and metals, in addition to OPs</td>
<td></td>
</tr>
<tr>
<td>Hancock</td>
<td>2008</td>
<td>FAMILY-BASED CASE-CONTROL STUDY OF PESTICIDE EXPOSURE AND RISK OF PD

EXPOSURE self-reported exposure; those who reported a direct application of any pesticide that was initiated prior to the reference age (age of onset for cases, age-at-examination minus</td>
<td><u>Strengths</u>
Since this is a family-based study, the positive association</td>
<td>The findings of this study present some limited evidence for an</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>mean disease duration among cases for controls) were classified as ever exposed, otherwise subjects classified as never exposed. Cumulative exposure calculated as frequency in days per year multiplied by duration in years.

HEALTH EFFECTS Most of the information in the paper relates to pesticides as a group, with only limited evaluation of specific functional types or chemical classes of pesticides, and is therefore not directly relevant to this review. Relevant information relating to OPs is given in the table below:

Ever use of OP insecticide and percentage of exposed cases and controls

<table>
<tr><th colspan="5">% exposed</th></tr>
<tr><th>Functional type</th><th>History of pesticide use for each chemical class</th><th>Cases, n=319</th><th>Controls, n=296</th><th>OR</th><th>95% CI</th></tr>
<tr><td rowspan="3">Insecticide, Organophosphorus</td><td>Ever</td><td>16.6</td><td>10.1</td><td>1.89</td><td>1.11-3.25</td></tr>
<tr><td>Ever to any other pesticide</td><td>46.1</td><td>39.5</td><td>1.53</td><td>1.05-2.23</td></tr>
<tr><td>Never</td><td>37.3</td><td>50.3</td><td>1.00</td><td>Referent</td></tr>
</table>
These results suggest a possible association between use of OPs and the risk of developing PD</td>
<td>is less likely to be confounded by unmeasured genetic and environmental influences

Family-based nature of the study might reduce recall bias, as unaffected relatives may be familiar with potential risk factors for disease

Family-based controls may be more motivated to participate, giving a higher participation rate

<u>Limitations</u>
The cases examined may not be representative of the general population of PD cases and the study design may not be generalisable

Family-based case-control offers less power than population-based case-control studies to detect effects, because of probable overmatching on exposure history</td>
<td>association between exposure to OPs and the occurrence of PD</td>
</tr>
<tr>
<td>Manthripragada</td>
<td>2010</td>
<td>STUDY EXAMINING POSSIBLE ASSOCIATIONS BETWEEN PD AND THE OPs DIAZINON,</td>
<td><u>Strengths</u></td>
<td>The study</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td>NOTE: this study is also discussed in our Polymorphisms section</td>
<td></td>
<td>
CHLORPYRIFOS AND PARATHION, AND THE INFLUENCE OF A FUNCTIONAL POLYMORPHISM AT POSITION 55 IN THE CODING REGION OF THE PON1 GENE

EXPOSURE Residential pesticide exposure estimated for each participant using residential history and a GIS (geographic information)-based system. The 1974-1999 average pesticide-specific pounds per acre used to categorise study participants as having zero, low or high residential exposure to each OP. Median value of pesticide exposure in controls used as cut-point between low and high categories.

HEALTH EFFECTS
Residential diazinon and chlorpyrifos exposures were highly correlated, with 81% of those ever exposed to chlorpyrifos also having been exposed to diazinon, and 70% ever exposed to diazinon also exposed to chlorpyrifos. The table below gives the results for association of residential exposure to chlorpyrifos, diazinon, and parathion with PD:

Residential exposure to specific pesticides and association with PD
<table>
<tr><th></th><th>Cases (n=351) No. (%)</th><th>Controls (n=363) No. (%)</th><th>Unadjusted OR (95% CI)</th><th>Adjusted OR* (95% CI)</th></tr>
<tr><td>Diazinon</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Zero**</td><td>149 (43)</td><td>184 (51)</td><td>1.00</td><td>1.00</td></tr>
<tr><td>Low</td><td>77(22)</td><td>90 (25)</td><td>1.06 (0.73-1.53)</td><td>1.02 (0.68-1.53)</td></tr>
<tr><td>High</td><td>125 (37)</td><td>89 (25)</td><td>1.73 (1.23-2.45)</td><td>1.55 (1.05-2.30)</td></tr>
<tr><td>Chlorpyrifos</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Zero**</td><td>170 (48)</td><td>215 (59)</td><td>1.00</td><td>1.00</td></tr>
<tr><td>Low</td><td>93 (27)</td><td>74 (20)</td><td>1.59 (1.10-2.29)</td><td>1.56 (1.06-2.31)</td></tr>
<tr><td>High</td><td>88 (25)</td><td>74 (20)</td><td>1.50 (1.04-2.18)</td><td>1.56 (1.02-2.40)</td></tr>
<tr><td>Parathion</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Zero**</td><td>185 (53)</td><td>198 (55)</td><td>1.00</td><td>1.00</td></tr>
<tr><td>Low</td><td>76 (22)</td><td>82 (23)</td><td>0.99 (0.69-1.44)</td><td>0.82 (0.54-1.21)</td></tr>
<tr><td>High</td><td>90 (26)</td><td>83 (23)</td><td>1.16 (0.81-1.66)</td><td>0.98 (0.65-1.48)</td></tr>
</table>
Notes: *adjusted for age (continuous), sex, ever-smoked, race, county, education (school years)
**reference category

The results suggest that exposure to chlorpyrifos, in both low and high exposure groups, was associated with an increased risk of PD; the authors further noted that the association seemed stronger among people ≤60 years of age with low or high exposure (for ≤60 years, zero vs. low/high- OR=2.65, 95% CI 1.19-5.90, for >60 years, zero vs. low/high- OR=1.43, 95% CI
</td>
<td>
Relatively large number of cases and controls

The GIS approach to estimating exposure is a semi-quantitative measure, and avoids problems of poor recall for historical and specific pesticide exposures

Data provided for specific OP compounds

<u>Limitations</u>
Some misclassification of exposure is still likely, due to incomplete address information and geocoding difficulties, and also factors that cannot be estimated, such as differences in wind patterns after pesticide applications, which would affect the amount of pesticide residue carried to nearby areas, or the amount of time individuals spent at home, or how pesticides were used
</td>
<td>provides some evidence that carriers of the variant MM PON1-55 genotype may be at increased risk of PD if exposed to the OPs diazinon or chlorpyrifos; no increase in risk was seen for parathion</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
0.98-2.11). For high exposure to diazinon the results showed increased ORs with borderline significance, and ORs for parathion with high exposure were less than 1.

The table below shows the results for the interaction between PON1 Leu-Met 55 and diazinon, chlorpyrifos, and parathion exposure in association with PD:

PON1 genotypes and Odds of PD associated with exposure to 3 specific OPs
<table>
<tr><th></th><th colspan="3">Zero Exposure</th><th colspan="3">Low/High Exposure</th></tr>
<tr><th></th><th>No. Cases/ Controls</th><th>Unadjusted OR (95% CI)</th><th>Adjusted OR* (95% CI)</th><th>No. Cases/ Controls</th><th>Unadjusted OR (95% CI)</th><th>Adjusted OR* (95% CI)</th></tr>
<tr><td>Diazinon</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>PON1-55</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>LL + LM</td><td>133/164</td><td>1.00**</td><td>1.00**</td><td>170/164</td><td>1.28 (0.93-1.75)</td><td>1.18 (0.83-1.68)</td></tr>
<tr><td>MM</td><td>16/20</td><td>0.99 (0.49-1.98)</td><td>1.00 (0.49-2.04)</td><td>32/15</td><td>2.63 (1.37-5.06)</td><td>2.24 (1.12-4.48)</td></tr>
<tr><td>OR (95%) for interaction</td><td></td><td></td><td></td><td></td><td>2.67 (1.09-6.55)</td><td>2.23 (0.89-5.62)</td></tr>
<tr><td>Chlorpyrifos</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>PON1-55</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>LL + LM</td><td>149/193</td><td>1.00**</td><td>1.00**</td><td>154/135</td><td>1.48 (1.08-2.02)</td><td>1.48 (1.04-2.12)</td></tr>
<tr><td>MM</td><td>21/22</td><td>1.24 (0.66-2.33)</td><td>1.17 (0.61-2.25)</td><td>27/13</td><td>2.69 (1.34-5.39)</td><td>2.61 (1.25-5.44)</td></tr>
<tr><td>OR (95%) for interaction</td><td></td><td></td><td></td><td></td><td>2.18 (0.89-5.31)</td><td>2.01 (0.80-5.01)</td></tr>
<tr><td>Parathion</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>PON1-55</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>LL + LM</td><td>157/177</td><td>1.00**</td><td>1.00**</td><td>146/151</td><td>1.09 (0.80-1.49)</td><td>0.90 (0.64-1.28)</td></tr>
<tr><td>MM</td><td>28/21</td><td>1.50 (0.82-2.75)</td><td>1.47 (0.78-2.75)</td><td>20/14</td><td>1.61 (0.79-3.30)</td><td>1.21 (0.57-2.60)</td></tr>
<tr><td>OR (95%) for interaction</td><td></td><td></td><td></td><td></td><td>1.07 (0.44-2.60)</td><td>0.94 (0.38-2.35)</td></tr>
</table>
Notes: *adjusted for age (continuous), sex, ever-smoked, race, county, education (school years)
**reference category

The above results suggest that, when examining the joint effects of PON1-55 and OP
</td><td>within the home

Low participation rate for controls (363 out of 822 eligible controls took part) could give rise to selection bias</td><td></td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>exposure, there was a 2-fold risk for carriers of the MM genotype exposed to diazinon, compared with persons with the wildtype or heterozygous genotype and no diazinon exposure (adjusted OR=2.24, 95% CI 1.12-4.48). Carrying the MM genotype and having been exposed to chlorpyrifos also increased the risk of PD compared with unexposed wildtype/heterozygous PON1-55 carriers (adjusted OR=2.61, 95% CI 1.25-5.44). The results for exposure to parathion and carrying the MM genotype do not suggest an increased risk of PD (adjusted OR 1.21, 95% CI 0.57-2.60)</td><td></td><td></td></tr>
<tr><td colspan="5">7.B.3. Cross-sectional studies</td></tr>
<tr><td>Salvi</td><td>2003</td><td>STUDY OF TOBACCO WORKERS CHRONICALLY EXPOSED TO OPs IN BRAZIL

EXPOSURE OP exposure history obtained via questionnaire. Plasma AChE levels of all subjects were within the normal range (3.2-9.0 U/l), and were not different between on- and off-exposure periods (4.7±0.9 and 4.5 ±1.1 U/l respectively). First evaluation performed while workers exposed to OPs; second evaluation conducted 3 months later, when workers had been off exposure for 3 months.

HEALTH EFFECTS Clinically significant extrapyramidal symptoms were present in 12 subjects out of the 25 who completed both evaluations. These subjects had a total score of parkinsonism of 5 or more in the ESRS (Extrapyramidal Symptom Rating Scale), which can be considered above the normal range and is unexpected in a group with mean age in years of 37.0 ±13.5. There was a considerable and statistically significant reduction of extrapyramidal symptoms after 3 months off exposure to OPs, but 9 subjects still had clinically significant symptoms as evidenced by a total score of 5 or more in the ESRS.

The table below indicates the scores on the ESRS:

Scores on the Extrapyramidal Symptom Rating Scale in tobacco workers during exposure to OPs and after 3 months of no exposure to OPs
<table>
<tr><th>Parameter</th><th>ON exposure (n=37)</th><th>(n=25)</th><th>OFF exposure (n=25)</th><th>Statistical analysis (p)</th></tr>
<tr><td>Parkinson questionnaire</td><td>5.46±4.25</td><td>5.64±4.40</td><td>3.04±3.74</td><td><0.001</td></tr>
<tr><td>Parkinson exam</td><td>5.14±5.64</td><td>4.72±5.72</td><td>3.04±4.17</td><td>0.056</td></tr>
<tr><td>Parkinson total</td><td>10.59±8.70</td><td>10.36±9.01</td><td>6.08±7.11</td><td><0.001</td></tr>
<tr><td>Akathisia</td><td>0.35±1.06</td><td>0.40±1.26</td><td>0.32±0.75</td><td>0.666</td></tr>
</table>
Notes: Akathisia is a movement disorder characterised by a feeling of inner restlessness and</td><td><u>Strengths</u>
Study uses the ESRS Scale, which is a clinical assessment tool

AChE levels available as measure of exposure

<u>Limitations</u>
Small sample size, and 12 of the original 37 were lost to follow-up

No unexposed group for comparison

Authors report frequent co-use by subjects in the study of at least one other chemical compound besides OPs</td><td>The study presents evidence that some subjects had continuing parkinsonian symptoms after exposure to OPs that endured after a period of no use of OP pesticides</td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		a compelling need to be in constant motion Data presented above are the mean score ±SD Statistical analysis refers only to the 25 subjects evaluated twice Clinically significant extrapyramidal symptoms were present in 12 subjects, evidenced by a total score of parkinsonism of 5 or more in the ESRS. This score can be considered above the normal range, especially in a relatively young population. There was a considerable and statistically significant reduction of extrapyramidal symptoms after 3 months without exposure to OP, but 9 subjects still had clinically significant symptoms. The *p* value for the questionnaire score indicates significance, and the exam score is on the borderline of significance. The total score has a value of <0.001		

Table 8: EXPOSURE SCENARIO AND PARKINSON'S DISEASE / PARKINSONISM

A. ACUTE EXPOSURE WITH CHRONIC EFFECTS

Table 8.A.2. Case-Control Studies

Test / Exposure	First Author and Date
	Das 2011
History of acute OP poisoning	+

B. CHRONIC LOW-LEVEL EXPOSURE

Table 8.B.1. Cohort Studies

Test / Exposure	First Author and Date		
	Albers 2004b	Engel 2001	Kamel 2007a
Unified Parkinson's Disease Rating Scale (UPDRS)	-	-	
Exposure to 10 specific OPs (chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, glyphosate, malathion, parathion, phorate, terbufos)			-
Exposure to any OP		-	
Exposure to azinphos methyl		-	
Exposure to diazinon		-	
Exposure to methyl parathion		-	

Table 8.B.2. Case-Control Studies

Test / Exposure	First Author and Date				
	Elbaz 2009	Firestone 2005	Gatto 2009	Hancock 2008	Manthripragada 2010
Exposure to OPs	-	-		+	
Unified Parkinson's Disease Rating Scale (UPDRS)				+	
Exposure to diazinon		-			
Exposure to malathion		-			
Exposure to parathion		-			
Exposure to diazinon in well water			+		
Exposure to chlorpyrifos in well water			-		

Test / Exposure	First Author and Date				
	Elbaz 2009	Firestone 2005	Gatto 2009	Hancock 2008	Manthripragada 2010
Exposure to dimethoate in well water			-		
Exposure to diazinon applied near home and in well water			+		
Exposure to chlorpyrifos applied near home and in well water			+		
Exposure to dimethoate applied near home and in well water			+		
Exposure to diazinon and carrier of MM genotype					+
Exposure to chlorpyrifos and carrier of MM genotype					+
Exposure to parathion and carrier of MM genotype					-

Table 8.B.3. Cross-Sectional Studies

Test / Exposure	First Author and Date
	Salvi 2003
Extrapyramidal Symptom Rating Scale (ESRS)	+

Table 9: OVERVIEW OF TESTS RELATING TO PARKINSON'S DISEASE / PARKINSONISM

This information is summarised here to aid members in interpreting the studies relating to Parkinson's Disease (PD) and extrapyramidal symptoms.

Test	Description
Unified Parkinson's Disease Rating Scale (UPDRS)	Since its introduction in 1987, the UPDRS has been used extensively by researchers and clinicians. Because of variability between scales and differences in weighting signs and symptoms, a committee chaired by Fahn was created in 1984 to develop a standardised scale (Fahn 1988). The UPDRS is a composite of previous scales and is now in widespread use. It is a tool for assessing and documenting the status of patients, and contains a detailed list of questions relating to physical signs and symptoms, mental and mood symptoms, indications for conducting a motor examination, and a means of staging and assessing the extent of disease (Factor et al, 2002). The original version of the scale assessed daily activities, motor skills and mental capacity, including behaviour and mood. When administering the scale, a neurologist observes a patient's performance and then scores performance from 0 (normal) to 4 (severe). Therefore, the higher the UPDRS score, the greater the disability from PD.
Extrapyramidal Symptom Rating Scale (ESRS)	The ESRS consists of 13 items and measures pseudoparkinsonian symptoms such as akathisia (restlessness and a need to be in constant motion), dystonia (a neurological disorder in which sustained muscle contractions cause twisting and repetitive movements or abnormal postures) and dyskinesias (involuntary movements such as tics). It is a physician-rated scale of extrapyramidal side effects, and was originally designed to measure these effects from antipsychotic medication (Chouinard and Margolese, 2005; Chouinard et al, 1980). It requires only a short time to complete the rating, approximately 10 minutes, and involves 6 questions about the patient's subjective experience of extrapyramidal features (slowness, stiffness, and tremor); a standardised procedure for physical examination, and 7 rater-assessed items that address parkinsonian features such as rigidity and tremor. The instrument may not differentiate effectively between dyskinesia and dystonia, however.

MMSE (Mini–Mental State Examination)	A commonly applied test of cognitive function. It is a brief 30-point questionnaire test used to screen for cognitive impairment, divided into subscores for orientation, attention, recall (short term memory) and language (Folstein et al, 1975). A score below 24 is used as a cut off for mild cognitive impairment. The MMSE is composed of 11 major items: temporal orientation (5 points), spatial orientation (5 points), immediate memory (3 points), attention/concentration (5 points), delayed recall (3 points), naming (2 points), verbal repetition (1 point), verbal comprehension (3 points), writing (1 point), reading a sentence (1 point), and constructional praxis (1 point). The MMSE has maximum score of 30, with five different domains of cognition analysed: (1) Orientation, contributing a maximum of 10 points, (2) Memory, contributing a maximum of 6 points, (3) Attention and calculation, as a measure of working memory, contributing a maximum of 5 points, (4) Language, contributing a maximum of 8 points, and (5) Design copying, contributing a maximum of 1 point. Any score greater than or equal to 25 points out of 30 is considered normal (intact). Below this, scores can indicate severe (≤9 points), moderate (10-20 points) or mild (21-24 points) cognitive impairment. The raw score may also need to be corrected for educational attainment and age.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF POLYMORPHISM AND ADVERSE EFFECTS

INTRODUCTION

1. The impact of polymorphisms on OP-related chronic toxicity was not considered in the 1999 COT report. The COT recommended that further research as to whether people with chronic disabling illness that is suspected of being related to OPs differ metabolically from the general population. A report of one investigation of polymorphisms of paraoxonase (PON1) was submitted as part of the Government funded research and considered in September 2007. The papers reviewed for this draft discussion paper report publications which summarise the evidence considered in the 2007 COT report and extend the analyses to include the polymorphisms associated with 'Dippers' flu'.

Metabolism of OPs

2. The metabolism of OPs is complex and biotransformation can lead to activation or detoxification of OPs. Metabolic activation can involve cytochrome P450 activation of phosphorothioate to the corresponding oxon metabolite (for example metabolism of diazinon to diazoxon). Detoxification can involve phosphoric acid triester hydrolases, carboxylesterases and glutathione redox system (Jokanović 2001). The balance between activation and detoxification depends on the genotype of individuals, the phenotype of the enzymes and also the specific OP under investigation. In particular, the polymorphism of Paraoxonase-1 (PON1) has been shown to be a major determinant of OP toxicity in animals for some OPs (e.g. diazinon) (Furlong et al 2000, Costa et al 2003, Li et al 2000). Thus, polymorphisms of PON-1 have been the focus of most studies investigating the association between OP-induced toxicity and polymorphisms.

Polymorphisims of Paraoxonase (PON1)

3. Serum paraoxonase hydrolyses a wide variety of substrates including OPs, arylesters, lipid hydroperoxide and lactones and has been suggested to have a role in diverse diseases such as atherosclerosis, microvascular disease in diabetes mellitus and Parkinson's Disease as well as a role in OP acute toxicity (Durrington et al 2001). The PON1 coding region has two polymorphisms involving the amino acids at position 55 (leucine [L] → methionine [M]) and 192 (glutamine [Q]→ arginine [R]), giving rise to isoenzymes which differ in their catalytic rate for the hydrolysis of OPs.

Investigations of PON1 Phenotype

4. Diazinon is the only OP currently used for sheep dip in the UK. The active oxon, diazoxon, can be detoxified by PON1. Hence, the influence of PON1 polymorphisms on diazoxonase activity is of interest. Davies and colleagues reported that diazoxon is metabolised slowly by the enzyme coded by the PON1-192 R allele compared to that coded by the Q allele. However, other OPs such as paraoxon are metabolised more slowly by the product of the Q allele. The assay used a pH of 8.5 and salt concentrations of 2M. Davies et al 1996 However, when O'Leary and colleagues investigated serum diazoxonase activity in 85 healthy volunteers using an improved assay (that utilised physiological conditions, pH7.4, 150 mM NaCl and 37^{o}C), they found that individuals with PON1 192 RR had the highest diazoxonase activity, with activity slightly reduced for QR genotypes and reduced further in QQ individuals (O'Leary et al 2005). Thus, these two studies gave opposing results regarding catalytic rate for the different PON1 enzymes towards diazoxon. In a follow up study, Richter and colleagues reported that the high pH/high salt assay was optimised with regard to the separation of PON1-192 phenotypes but was not optimised to evaluate *in vivo* rates of OP detoxification. Richter cautioned against interpreting lower activity of PON1 192 R at high salt concentration to indicate that PON1-192 RR homozygotes would be more sensitive to diazinon (Richter et al 2009). In this study they reported that the two PON1-192 isoforms had similar catalytic efficiencies for hydrolysing diazoxon. In separate *in vivo* studies using PON1-knockout mice, Li and colleagues reported that both isoforms of PON1-192 conferred a similar degree of protection to diazoxon induced toxicity. In *in vitro* studies, these authors found that both isoforms hydrolysed diazoxon at a similar rate at physiological salt concentrations but at high salt concentrations (2M NaCl), PON1-192 Q dioxonase activity was stimulated whereas PON1-192 R was inhibited (Li et al 2000).

Studies Considered

5. The authors of polymorphism studies in sheep dippers have specifically reported phenotype profile for diazoxonase activity using the method of Davies et al (i.e. they found individuals with PON1-RR to have the lowest diazoxonase activity). Reaching a conclusion on these data is critical for interpreting the results of PON1 polymorphism studies of the health effects of OPs.

6. Most studies relate to polymorphisms of PON1 in sheep farmers with one study also investigating the genotype of other metabolising enzymes (such as CYP450, GSTP1, GSTT1 and GSTM1) in sheep farmers. There were 5 case-control studies from the Centre for Occupational and Environmental Health, University of Manchester investigating PON1 genotype and phenotype and risk of chronic ill health in sheep farmers (as defined by symptomatology including one study of 'dippers' flu'). A separate cross-sectional study of US farm workers applying pesticides investigated PON1 genotype and symptoms of chronic toxicity.

7. A case-control study of PON genotype and association with Parkinson's Disease in subjects residing near to agricultural land sprayed with OPs was retrieved. A cross-sectional study investigated PON1 genotype and the occurrence

of EEG and brain tomography in an agricultural community with regular exposure to OPs was reviewed.

8. No studies investigating the possible associations between genotypes and neuropsychological and psychiatric outcomes in OP-exposed populations were retrieved

9. During the review of papers identified, it was noted that there may be additional studies of PON genotypes and other aspects of pesticide exposure, which have not been retrieved (for example Hernandez et al 2003). This study reported on polymorphisms in green house sprayers, some of which reported exposure to OPs, but did not investigate health effects in pesticide-exposed workers and hence was not included in the current review.

STUDIES OF SHEEP FARMERS (UK)

Paraoxonase (PON1) Polymorphisms in Farmers Attributing ill Health to Sheep Dip

10. Cherry et al 2002, undertook a case-referent study where 175 subjects who attributed their chronic ill health to sheep dip were recruited by radio and newspaper advertisement (Cherry et al 2002). Of these, 102 cases identified one or more eligible referents who dipped sheep and were thought to be good health, were of similar age (within 10 years) and whose sheep dipping activity was similar to the case subject. 309 referents were nominated, of which 234 were included in the study. A nurse visited cases and referents at home between July 1999 and May 2000 and obtained information on exposure and took a venous blood sample. PON1 genotypes for the 55 and 192 polymorphisms were determined (Annex 10, Table 6.B.2.). The ORs associated with polymorphisms (409 subjects) and diazoxonase (above or below the median for 379 subjects) were estimated by logistic regression. The time spent dipping by cases (19.4 (SD 7.5) years) was significantly less than referents (22.0 (SD7.2) years) (p=0.0004). Two-thirds of participants reported that at some time they had used dips containing diazinon (115 (66%) cases, 152 (65%) referents).

11. In both cases and referents linkage disequilibrium was observed; all subjects with PON1-192 RR also had LL at position 55. Cases were less likely than referents to be homozygous for QQ at position 192 (69 (39%) cases, 140 (60%) referents and were more likely to be homozygous for LL at position 55 (86 (49%) cases, 74 (32%) referents). Serum diazoxonase was below the median in 58% of (95) cases and 44% (94) referents. Hydrolytic activity was linked with polymorphism at position 192 [participants below median QQ (36%, (69)), QR (61% (97)) and RR (85% (23)) p<0.0001]. The OR for case status was significantly increased for PON192-QR or RR versus QQ 2.25 (95%CI 1.49-3.42) (p=0.001). The OR for case status was significantly increased for PON1-55 LL versus LM or MM 1.92 (95%CI 1.26-2.93). The authors reported that the interaction term was not significant (p=0.75) suggesting that the effects of the two polymorphisms were independent (Annex 10, Table 7.B.2.).

Paraoxonase and Susceptibility to OP Poisoning in Farmers Dipping Sheep

12. This case-referent study investigated serum paraoxonase activity to a range of substrates using the same cases and referents (Mackness et al 2003). Serum PON1 activity to paraoxon, phenylacetate and diazoxon were measured. The methods for serum paraoxonase measurements were based on methods previously published by the authors and in the case of diazoxonase was based on Davies et al (paragraph 3 above) (Table 6.B.2.). The ORs for genotypes were reported for participants who used diazinon. The OR for PON1 192 QR or RR versus QQ was 2.39 (95% CI 1.46-3.98) and for position 55 LL versus LM or MM was 3.16 (95% CI 1.88-5.31).

13. There were no significant differences between cases and controls for paraoxonase activities. However, when the two groups were divided into quintiles according to serum diazoxonase activity, farmers in the lowest quintile had a greater risk of being a case (OR 2.47 (95%CI 1.23-2.82)) than those in the other 4 quintiles. A similar analysis for paraoxon and phenylacetate revealed no significant differences in ORs between quintiles of serum activity. Thus for diazoxonase PON1-192 QQ homozygotes had the highest activity, QR heterozygotes intermediate activity and RR homozygotes the lowest activity. This pattern was reversed for paraoxon. The rate of serum hydrolysis of paraoxon was greatest in cases and controls with the R/L haplotype (both $p<0.001$). These results are consistent with the pattern of paraoxonase activity for different polymorphisms of PON1 reported in the previous study (paragraphs 10 and 11) (Annex 10, Table 7.B.2.).

Paraoxonase Polymorphisms and Self-Reported Chronic ill-Health in Farmers Dipping Sheep

14. This case-referent study reported on detailed health questionnaires sent to the cases and referents used in the previous studies (Povey et al 2005). The visiting nurse reviewed the questionnaire with the farmer and collected additional information on exposure. Consent to approach the general practitioner was sought from each respondent. GPs were asked whether there was any history of neurological disease that could be confused with effects of OPs. A detailed 95 symptom questionnaire was completed and subjects who provided usable answers to at least 90 questions were included in the main study. The authors used a stepwise approach to exclude subjects firstly on clinical grounds and subsequently on the results of a discriminant analysis which excluded subjects on the basis of atypical symptoms. Subjects fell into four groups. Group 1 comprised subjects with chronic condition attributed by GP to a defined event. Group 2 comprised subjects with established neurological disease. Group 3 comprised subjects identified by discriminant analysis as having atypical symptoms and subjects in group 1. Group 4 comprised subjects in groups 1 and 2 and also those identified by discriminant analysis as having atypical symptoms. A five staged analysis was undertaken; stage 1 all subjects excluding those with less than 90 usable symptom answers, stage 2 all subjects excluding group 1, stage 3 all subjects except groups 1 and 2, stage 4 all subjects excluding groups 1 and 3, stage 5 all subjects excluding groups 1,2, and 4 (Annex 10, Table 6.B.2.).

15. Cases were more likely than referents to report that they had been troubled by ill-health during the past month and more likely than referents to report their health had been affected by sheep dips. A total of 20 subjects had a chronic condition diagnosed by their GP (eight were cases). A total of 21 subjects had established neurological disease (19 cases). After exclusion of group 1, discriminant analysis correctly identified 83.8% of cases. 61 subjects were in group 3, (42 cases predicted as referents and 19 referents predicted as cases). After exclusion of subjects in groups 1 and 2 discriminant analysis correctly identified 86.8% of cases. The risk associated with QR or RR genotype remained elevated (>2-fold) when compared to QQ genotype even after exclusion of subjects on clinical grounds and after discriminant analysis. Risk associated with QR genotype (versus QQ) remained similar, but that of RR was reduced when subjects were excluded by discriminant analysis. The risk associated with PON1 192 LL genotype remained elevated (OR 1.79-2.15) compared to LM and MM genotype even after subject exclusion. The authors concluded that subject exclusion to provide a more homogeneous case and referent population made little difference to the risk associated with PON1 genotype suggesting the original analysis (paragraphs 10 and 11) was robust (Annex 10, Table 7.B.2.).

GST, CYP and PON1 Polymorphisms in Farmers Attributing ill-Health to OP Sheep Dip

16. This case-referent study reported on genotyping data for GSTP1, GSTM1, GSTT1 as well as genotyping and phenotyping for PON1 using the same cases and referents in the previous studies summarised above (Povey et al 2007b). Genotyping for GSTM1*1/*2, GSTT1*1/*2, GSTP1*A/*B/*C, CYP2D6*3/*4, CYP3A4*1A/*1B CYP3A5*1/*3. CYP2D6*3/*4, was defined by identifying CYP2D6*3 and 2D6*4 alleles. CYPD2D6 wildtype was identified as having no *3 or *4 allele. CYP2D6 heterozygotes as having one *3 or *4 allele and CYP2D6 homozygotes as having two *3 or *4 alleles or one *3 or *4 allele. CYP2D6 HOMs were classified as poor metabolisers (Annex 10, Table 6.B.2.). There were no significant differences in the frequency of GSTM1*2 (null) genotype and GSTT1*2 (null) genotype in cases and referents in either whole population or in only those who were exposed to diazinon. Restricting the number of subjects by applying the stages of the discriminant analysis (outlined in para12) did not affect these results. In the whole population, there was an elevated but non-significant risk associated with combined GSTM1 and GSTT1 null genotype (9% of cases, 5.8% of referents were double-null homozygotes (OR= 1.61 (95%CI 0.74-3.48)). In the staged analysis, there was evidence of an increased risk after exclusion of subjects on clinical grounds. It was found that 10.4 % of cases were double null homozygotes at stage 3 compared to 5.4% of referents (OR 2.06 (0.85-5.04)). A similar result was reported in restricted analysis of subjects exposed to diazoxon (OR = 2.60 (0.72-10.42)). With regard to GSTP1, the risk of having a *B or *C allele was slightly increased in the whole study population and in those exposed to diazinon but none of the reported ORs reached significance (Table 7.B.2.).

17. The risk associated with CYP2D6 wild type (defined using CYP2D6*3 and CYP2D6*4) was elevated but not significantly (OR 1.47 (95%CI 0.83-2.60)). Restricting the population using the staged assessment did not alter the results. There were no significant differences in the distribution of CYP3A4 and 3A5

genotypes between cases and referents. Subjects homozygous for CYP3A4*1B or CYP3A5*3 were rare with only one of each identified in this population. The CYP3A4*A/*B allele frequency did not differ between cases and referents (0.975/0.025 and 0.964/0.036 respectively). CYP3A5*1*3 allele frequency did not differ between cases (0.957/0.043) and referents (0.942/0.057) (Annex 10, Table 7.B.2.).

18. The OR (95%CI) for PON1 QR/RR polymorphisms was 5.75 (1.22-29.11) in subjects characterised as either CYPD2D6 HET or CYPD2D6 HOM and 1.81 (0.99-3.38) in CYPD2D6 WT subjects. For PON1 LL the OR (95%CI) was 3.98 (2.08) for CYPD2D6 WT and 1.11 (0.23-4.98) for CYPD2D6 HET/HOM. For GSTP1 there was an increased OR for all combinations except *A*A (see table below). There were no increased ORs for PON1 polymorphisms combined with GSTT1 or GSTM1 except for and association between PON1 LL with GSTT1*1 (4.08 (2.15-7.76) (OR for GSTT1*2 was1.21 (0.29-5.16)). The authors hypothesised that risks associated with CYP2D6 WT would be greater than CYP2D6 HET/HOM. The risk was increased in PON1-55 LL subjects with CYP2D6 WT. However, increased risk associated with low diazoxonase activity and PON1-192 QR/RR genotype was observed in individuals with lower CYP activity (CYP2D6 HET/HOM). These results were not consistent with the original hypothesis. The authors felt the results could be due to misclassification of the CYP 2D6 genotype or by chance (Annex 10, Table 7.B.2.).

19. The authors concluded that further studies were needed to investigate pathways of OP activation and detoxification and whether GSTP, GSTT, GSTM genotype affect diseased risk in population exposed to OPs.

Dippers' Flu' and its Relationship to PON1 Polymorphisims

20. This case-referent study reported on the assessment of 'dippers' flu' and its association with PON1 genotype using the same cases and referents as in previous studies (Cherry et al 2011). Subjects were asked whether they had ever had dippers' flu in the questionnaire completed prior to interview. This information was expanded by the nurse in discussion with the subject and information on symptoms and dates was collected. Subjects provided information on dipping practices and symptoms for each year from 1970 to 2000. For each subject, a report of 'dippers' flu' at any point in the interview and date of first report (from 1970 onwards) were recorded. (OP pesticides were generally not used prior to 1970) No definition of 'dippers' flu' was offered and in subjects who were unsure if the symptoms were properly considered as 'dippers' flu' the answer was considered positive if he reported symptom details (Annex 10, Table 6.B.2.).

21. The study population was recruited as in Cherry 2002 (paragraph 8). Information on possible dippers flu was expanded during nurse interview. (referred to in paper as chronic group). Of the 175 cases, 116 reported they had experienced 'dippers' flu'. Of the 234 referents (referred to as no chronic illness or not chronic), 42 reported that they had at some point experienced 'dippers' flu'. The relationship of 'dippers' flu' to PON1 polymorphisms and diazoxonase activity was investigated. In addition, the interaction between genotype and handling concentrate on 'dippers' flu' was examined. A case-crossover analysis was undertaken. Thus, for subjects

who had started dipping after 1970 and who developed dippers flu in a year immediately following a dipping year with no symptoms, answers in each of the 2 years (one with dippers flu, the preceding one without) were extracted for more detailed case-crossover analysis of precipitating factors. A survival analysis was undertaken (time taken from first dipping to onset of 'dippers' flu').

22. In the total group of chronically ill farmers, and also those who started after 1969, none of the indices of sheep dip were related to the presence of ever/never of 'dippers' flu'. In those without chronic ill health, the cumulative number of times pouring a concentrate was greater in those reporting dippers flu. In a further analysis comparing those ever handling with those never handling concentrate, in those who started dipping after 1969, an association with dippers flu was reported. Subjects with start date after 1970 had very similar results except the difference between those with 'dippers' flu' and those without 'dippers' flu' assessed for total concentrate handling was not significant. (2213.2 (6032.9) with 'dippers' flu' and 1422.3 (4712.1) without 'dippers' flu' p value 0.50. In a further assessment those ever handling concentrate compared with those never handling concentrate in the non chronic group who started dipping after 1969 were more likely to have 'dippers' flu' if they had ever handled concentrate (concentrate 'dippers' flu' (21/89, 23.6%) verses no concentrate (2/28, 7.1%) p=0.04 (annex 10, Table 7.B.2.).

23. The occurrence of LL at position 55 was not related to 'dippers' flu' in any of the four study groups (all with chronic illness, all with chronic illness stating dipping after 1970, all with no chronic illness, and those without chronic illness who stated dipping after 1970). In chronic illness group, those with two R alleles were at more risk than those with only one R allele (OR 2.04 (95%CI1.08-3.87)). In those without chronic illness the same pattern of results were found but the number of subjects with RR was small. A significant increase in OR was reported for the not chronic group who started dipping after 1970 (OR 2.52 (95%CI 1.00-6.37). Analysis of diazoxon hydrolysis was lower in those with R allele. Mean diazoxon hydrolysis was lower in those with dippers flu (not significant).

24. Exposure was redefined to include only those who had handled diazinon concentrate (pouring concentrate or adding it to the dipping bath) compared to all other subjects. Additionally subjects reported by family doctors to have neurological deficits (n=20) or diagnosed illnesses with neurological symptoms (n=22) were excluded. In univariate analysis, the OR for the interaction is greater than that of either of the main effects in each study group suggesting those who handled concentrate and had an R allele were at greater risk of developing dippers flu. In the multivariable analysis with both main effects and interaction in the model, this size of interaction effect was greater than in the univariate analyses in those who were chronically ill. The authors suggested the risk of 'dippers' flu' was largely confined to those who handled concentrate and had the susceptibility allele. The multivariable analyses of those who were healthy in the original study showed a reduction of size of the interaction term when both main effects were included in the model; the estimated risk associated with interaction was still >1.00 but the authors thought this might be due to chance (Annex 10, Table 7.B.2.).

25. There was no evidence in the cross-over analysis for an association of ill-health with the susceptibility allele (but this assessment was based on only 43 cases

31 from the chronic group and 12 from the group with no chronic illness). The time taken from first dipping to development of 'dippers' flu' was calculated for those starting to dip after 1969 (date of last dipping without 'dippers' flu'). In chronically unwell series there was no relationship with latency (RR 1.02 (95% CI 0.57-1.81) In the series without chronic illness, the ratio was raised (RR 2.49 (95%CI 1.03-6.02) with those having an R allele at position 192 having a less favourable survival curve (figure reproduced in article). The six subjects with two R alleles had a slightly larger relative risk (2.74) than those with one R allele but this difference did not approach significance (Table 7.B.2.).

26. Overall, the authors concluded that 'dippers' flu' and chronic ill-health attributed to dipping share a common polymorphism (R allele of POPN1-192). The interaction between handling concentrate and PON1 genotype supports the conclusion that OP may cause 'dippers' flu'.

Comment: PON1 Polymorphisms in Sheep Dippers Exposed to OPs.

27. These studies were all limited in that the case definition was based on self-report of chronic illness. The authors report the symptoms which best discriminated cases from controls were difficulty in concentrating and muscle spasm. It was therefore difficult to subdivide the case group into severe and less severe cases. However, the genotype status would not have been known to study participants at the time of recruitment and interview. The critical aspect of these studies concerns the biological logic used to associate PON1 192-R genotypes with increased risk of chronic illness. The authors reported that PON1-192 R allele confers a slower rate of diazoxon detoxification and hence would be consistent with increased risk of chronic toxicity. All the diazoxonase measurements were based on the method of Davies which used non-physiological conditions. However, if the PON1-192 R allele is associated with the fastest rate of diazoxon detoxification then there would be no rationale to associate this genotype with chronic toxicity.

CROSS-SECTIONAL STUDY OF PON 1 IN SOUTH AFRICAN FARM WORKERS

28. A total of 347 subjects (68% of eligible farm workers), from 75 participating farms (65% of eligible farms) were recruited (Lee et al 2003). Of these, 100 were selected by convenience and had blood samples drawn towards the end of the study for PON1 genotyping. Of the 100 subjects selected, 49 were pesticide applicators and 51did not apply pesticides. Details of pesticides used were not provided, although it is stated that OPs were used. Symptoms had to be present within 3 months of the survey (Annex 10, Table 6.B.3.). Following symptoms were compatible with chronic toxicity (considered consistent with chronic OP toxicity), chronic abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbances, limb numbness, paraesthesia, limb pain, limb weakness. Subject scores of a point for each symptom: ≥2 categorised as having toxic symptoms, ≤1 not having toxic symptoms. This divided the population in half. As expected the proportion of subjects with toxic symptoms was significantly higher among applicators than non-applicators of pesticides (63.3% v 37.3%, OR=2.9 (95%CI 1.3-6.5, p=0.009). Subjects with Arg/Arg (R/R) (PON1-192 R.R) were

classified as fast genotype (n=37). Those with Gln/Arg (Q/R) and Gln/Gln (Q/Q) were combined as slow genotype (n=45, 60%) (Annex 10, Table 7.B.3.).

29. A significantly higher proportion of subjects with the slow genotypes (PON1-192 Q/R or QQ) reported two or more symptoms of chronic toxicity compared to those with fast PON activity (OR 2.8 (95%CI 1.1-6.7)). The proportion of subjects with toxic symptoms increased in a step-wise fashion from non-applicator-fast genotype (15%), non-applicator-slow-genotype (42.9%), -applicator -fast genotype (58.8%), applicator-slow-genotype (75%) (Mantel-Haenszel Trend test p=0.001). In multivariable logistic regression analysis, the independent predictors of chronic pesticide toxicity were previous history of head injury (2.8 (95%CI 1.7-4.7), slow genotypes (2.9 (1.7-4.9) and history of having worked as a pesticide applicator (OR 5.1 (95%CI 3.2-8.9)) (annex 10, Table 7.B.3.).

30. The results of this small study report a genotype for susceptibility for chronic OP toxicity which are opposed to the results of published studies in sheep dippers. Paraoxonase activity was not measured in this study. The nature and extent of OP exposure were not reported which would have been important in assessing the potential influence of genotype on toxicity.

RELATIONSHIP OF EEG AND LORETA TOMOGRAPHY TO PON1 STATUS IN OP EXPOSED RURAL COMMUNITY

31. Browne et al 2006 undertook a small cross-sectional study of 30 subjects from a rural community (Israel) where OP pesticides were regularly used (Browne et al 2006). Controls were taken from urban areas with no history of exposure to anticholinesterases agents, no know neurological or psychiatric conditions. Biochemical data were available for 91 controls. Data from EEG measurements, and Low Resolution brain electromagnetic tomography (LORETA) reported (Table 6.B.3.). PON1 and arylesterase were significantly higher in exposed 447% and 441% of control p<0.001. (These authors used an assay based on a publication by Furlong et al which used a pH of 8.5 and 0.5M NaCl using paraoxon as the substrate.) This particular result is not consistent with the results reported by Cherry et al 2011 which reported a slight but not statistically significant increase in PON1 activity in referents compared to OP-exposed sheep farmers (Annex 10, Table 7.B.3.).

32. With regard to PON1-Q192R (CAA to CGA) substitution (R allele), in controls there was a gene-dependent increase in PON1 correlated with R allele (PON activity nmol/min*ml) QQ 29.6, QR 71.3, RR 99.9. slope35.18, R^2 0.9886). The authors reported more robust changes in exposed subjects QQ 150.7, QR 384.0, RR 513.7, slope 181.48, R^2 0.9735. The authors controlled for effect of PON L55M substitution by selecting individuals without the M allele and reported R allele associated changes in PON1 activity remained (Annex 10, Table 7.B.3.).

33. The authors hypothesised that increased beta3 EEG activity may differ among exposed individuals with different genotype profiles. Significantly increased beta3 activity in frontal cortical regions and decreased activity in the temporal regions in exposed individuals with the R allele compared to controls and exposed without the

R allele. Comparing LORETA values (representing source densities) in these brain regions revealed exposed with R allele exhibited significant frontal activity and decreased temporal activity (P<0.03). Non-exposed and exposed without R allele had similar LORETA patterns.

34. The authors noted that upregulation of PON1 activity and alterations in brain activity following OP exposure might seem to contradict one another. It was suggested that prolonged activity might be deleterious over the long-term. Furthermore, It was suggested that over-expression might have a negative influence on brain structure and function. However, overall the authors did not provide a coherent rationale as to why increased paraoxonase activity could be related to the deleterious effects reported.

STUDY OF PON1 POLYMORPHISM (PON1-55) AND OCCURRENCE OF PARKINSON'S DISEASE (PD)

35. Manthripragada et al undertook a case-control study of PON1 polymorphisms and Parkinson's Disease (PD) (Manthripragada et al 2010). Cases and controls were recruited between 1 Jan 2001-1 Jan 2008. Of the 1167 cases invited, 563 were eligible (PD diagnosis no longer than 3 years before recruitment, sufficiently healthy to be examined, currently residing in one of three counties, residing in california for at least 5 years) Of these, 473 cases were considered eligible by UCLA movement disorder specialist who confirmed clinically probable or possible PD. The final group of 351 cases were included for assessment. For controls, a random sample from Medicare enrolees and from homes identified at random from tax assessor housing unit data listed on residential parcel maps. A total of 1297 subjects were contacted. Eligibility included, not having PD, being at least 35 years, current residence in one of three counties of interest, residing in California for at least 5 years prior to recruitment, not being too ill for interview. One person per parcel unit allowed to enrol. Of 822 eligible controls, 414 did not participate (declined or moved from area prior to interview), 5 did not provide adequate demographic data, unable to estimate pesticide exposure for 15, no DNA sample for 24, and one sample failed during genotyping. There were 363 controls included in the study (Annex 10, Table 6.B.2.).

36. Residential exposure was calculated from residential history and a GIS-based system that combined pesticide use reporting data and land use maps. A time specific average exposure was calculated for each subject. This was derived from period specific average of all pesticide applications separately for diazinon, chlorpyrifos and parathion and the group of all other OPs (n=64) applied in California agriculture between 1974-1999.

37. The authors reported a 2-fold risk for diazinon exposed subjects with MM genotype compared with persons with wild type or heterozygous or no diazinon exposure (OR = 2.24 (95%CI 1.12-4.48)). This risk increase was evident among white people alone (2.68 (1.23-5.83)) even when adjusting for other OPs (2.43 (1.04-5.15). For those highly exposed to diazinon, an even larger increase in risk for MM carriers was reported (OR=5.30 (1.71-16.4)) and a strong interaction on the multiplicative scale (OR=4.59 (1.37-15.4)). The association was stronger when the

assessment was restricted to white people only, based on small number of highly exposed (zero/low versus high OR=9.1 (2.0-41)). Effect estimates were similar for those ≤60 and >60 years. (Annex 10, Table 7.B.2.).

38. Carrying MM genotype and exposed to chlorpyrifos was associated with an increase risk of PD compared with unexposed wild type/heterozygous PON1-55 carriers (OR=2.61 (1.25-5.44), white only (2.95 (1.31-6.64)). The effect was slightly reduced when adjusted for other OPs (among whites 2.76 (1.16-6.15)). There was a slight increase in risk in LL and LM carriers (1.48 (1.04-2.12), whites only (1.48 (1.00-2.17). There was a stronger association in subjects ≤60 years (unadjusted OR=5.30(1.17-16.4)) (OR adjusted for other OPs=6.14 (1.34-28.05)); OR for >60 years adjusted OR=2.36 (0.97-5.79)).

39. No associations between parathion and PD even for exposed PON1-55 MM genotypes carriers.

40. This study provided polymorphism (PON1 55) data for residents exposed to OPs, with objective measure of exposure. The authors noted poor response of controls and possible selection bias. However, this was thought unlikely to affect PON1-55. Possible misclassification of exposure due incomplete address, geocoding difficulties, differences in wind patterns during/after pesticide applications, amount of time spent at home, introduction of pesticides into the home. Overall, the authors concluded that OP exposure was associated with an increased risk of PD in subjects carrying PON1-55 MM genotype. This is consistent with evidence suggesting reduced metabolism of oxon OP metabolites by these subjects.

DISCUSSION AND CONCLUSIONS; POLYMORPHISMS

41. There are relatively few studies of genotype-environment interaction. Most studies relate to PON1 genotype and phenotype in OP exposed populations (predominantly sheep dippers in the UK). In all of the studies where paraoxonase activity was measured the methods utilised high pH and high salt concentrations. There is considerable uncertainty regarding the adequacy of these methods to predict *in vivo* paraoxonase activity. The key attributes and findings of the studies reviewed are given below.

Study	**Exposure and Health effect investigated**	**Genotype associated with health effect**	**Phenotype associated with health effect**	**Conclusion**
Cherry (2002) Case-referent 175 cases, 234 referents	Sheep dippers (UK) exposed to OPs. Cases with self-reported chronic ill health	PON1 192 RR or QR and PON1-55 LL significantly associated with case-status	Serum diazoxonase significantly lowered in individuals with QR and RR genotypes	Uncertainty as to whether case status with R genotypes was associated with low or high diazoxonase activity
Mackness (2003) Case-referent 175 cases, 234	Sheep dippers (UK) exposed to OPs. Cases with self-reported chronic ill health	PON1 192 RR or QR and PON1-55 LL significantly associated with	Serum diazoxonase significantly lowered in individuals with	Uncertainty as to whether case status with R genotypes was associated with

Study	Exposure and Health effect investigated	Genotype associated with health effect	Phenotype associated with health effect	Conclusion
referents		case-status	QR and RR genotypes and serum paraoxonase increased in QR/RR subjects.	low or high diazoxonase activity. The finding of increased paraoxonase activity with R genotype is consistent with other published studies.
Povey (2005) Case-referent Full 175 cases Stage 1 169 Stage 2 161 Stage 3 142 Stage 4119 Stage 5 107 Full 234 referents Stage 1 228 Stage 2 216 Stage 3 214 Stage 4 197 Stage 5 202	Sheep dippers exposed to OPs. (UK) Cases with self-reported chronic ill health. A stepwise exclusion of subjects with chronic neurological conditions and a discriminant analysis to exclude subject with symptoms not conforming to case definition	PON1 192 RR or QR and PON1-55 LL significantly associated with case-status. Results unaffected by exclusion of subjects to confine analysis to a more homogenous case-definition	Serum diazoxonase activity not reported	PON1 192 RR or QR and PON1-55 LL significantly associated with case-status
Povey (2007) Case-referent 175 cases, 234 referents	Sheep dippers(UK) exposed to OPs. Cases with self-reported chronic ill health	GSTP1, GSTM1 and GSTT1 genotypes examined	Not reported	No clear association between these genotypes and case status
Cherry (2011) Case-referent 175 cases, 234 referents	Sheep dippers (UK) exposed to OPs. Cases with self-reported 'dippers'flu'	PON1 192 RR or QR significantly associated with case-status. PON1-55 LL not associated with case-status	Serum diazoxonase was lower in those with R allele. Mean diazoxon hydrolysis was lower (not significant) in those with dippers flu.	Uncertainty as to whether 'dippers'flu' in subjects with R genotypes was associated with low or high diazoxonase activity
Lee (2003) Cross-sectional 49 pesticide applicators/51 non-applicators	South African farm workers (exposure to OPs stated but no details given) Symptoms reported to be consistent with chronic OP toxicity.	Significantly higher proportion of subjects with slow genotypes (PON1 192 QQ or QR) reported two or more symptoms of chronic (OP)	Not reported	This small study gave opposite results to those reported in sheep dippers in the UK

Study	Exposure and Health effect investigated	Genotype associated with health effect toxicity	Phenotype associated with health effect	Conclusion
Browne (2006) Cross-sectional Genotyping of 30 exposed and 91 controls.	Rural community (Israel) OPs regularly used. EEG and brain tomography investigated.	Significantly increased beta3 activity in frontal cortical regions and decreased activity in temporal regions associated with PON1-192 R allele Significant changes in brain tomography in these regions associated with PON1-192 R allele	PON1 paraoxonase activity significantly increased (4x) in exposed subjects.	Uncertainty regarding the method of paraoxonase measurement. Overall authors did not give a coherent rationale as to why increased paraoxonase could be related to the deleterious effects reported.
Manthripragada (2010) Case-control 351 cases/361 controls.	Case-control study of rural community (US) with residential exposure assessed by GIS system Association with Parkinson's Disease (PD) investigated.	PON-1 MM genotype associated with exposure to diazinon or chlorpyrifos gave an approximate 2-fold increase in risk for Parkinson's Disease (PD)	Not reported	The observed associations would be consistent with lower paraoxonase activity in subjects with PON1-55 MM genotypes.

42. The studies undertaken in sheep dippers from the UK who predominantly use diazinon to dip sheep provide consistent evidence for an association between PON1-192 RR genotype and exposure to OPs and evidence of self-reported chronic toxicity. The rationale presented in these publications is that PON-192-RR genotype is associated with the lowest hydrolysis of diazinon. However, there is considerable uncertainty as to whether diazoxon hydrolysis *in vivo* is lowest in subjects with PON1 192 RR genotype compared to other PON1 192 genotypes (QR and QQ). O'Leary and colleagues used a diazoxonase assay that utilised near physiological conditions and reported that individuals with PON1 192 RR had the highest diazoxonase activity.

43. The small study in South African farm workers gave opposing results to the studies in sheep dippers with regard to PON1 genotype and association with symptoms of chronic OP toxicity. There was no measurement of paraoxonase activity in these subjects. No clear conclusions can be reached.

44. No clear conclusions can be reached with regard to the small study of EEG and brain tomography. The authors had no clear rationale as to why increased paraoxonase activity observed in exposed subjects in this study could be associated with the effects on EEG activity and brain tomography.

45. One case-control study reported an association between Parkinson's Disease and PON1 55 MM genotype. This was assumed to be consistent with lower paraoxonase activity in subjects with PON1-55 MM but no measurements of paraoxonase activity were reported. Dick and colleagues reported a gene-environment association for PD (solvents/GSTM1) but no significant association for pesticides/PON1 and PD (OR 4.43 (0.88-22.31 for PON L55M) (Dick et al 2007).

Table 6: POLYMORPHISM STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
A. Acute Exposure with Chronic Effects									
6.A.1. Cohort studies - none									
6.A.2. Case-control studies - none									
6.A.3. Cross-sectional studies - none									
6.A.4. Case series studies - none									
6.A.5. Case reports - none									
B. Chronic Low-Level Exposure									
6.B.1. Cohort studies - none									
6.B.2. Case-control studies									
Cherry	2002	UK	197 volunteers recruited by radio and newspaper advertisement as people who dipped sheep in the Uk and who were ill and believed this was because of exposure to sheep dip. (nine used in pilot study, four refused/could not be contacted, six had not dipped sheep and three died before interview). 175 cases interviewed	Cases asked to name up to three, who were not blood relatives, who dipped sheep and were in good health, lived near to case and were of similar age. Of 175 cases, 102 identified one or more eligible referent (309 in total). Of these 11 were not eligible because they had not dipped sheep, 39 did not match any case (by age, location or were	Nurse visited between June 1999 and May 2000 and obtained information on exposure and health and took a venous blood sample. PON1 genotypes determined by PCR amplification and digestion by restriction enzymes. Hydrolysis of diazoxon measured (in 379 subjects, 93%) . (Diaoxonase method not reported but probably same as Mackness 2003 and Cherry 2010)	Polymorphism at 192 position, RR, QR, QQ Polymorphism at 55 position' LL, LM, MM	Qualitative assessment of duration of dipping and use of diazinon dips.	Not reported	Odds ratios associated with polymorphisms and diazoxonase activity (above or below median) assessed by logistic regression.

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
				blood relatives) and were not approached, and 25 were approached but refused to participate. . Overall 234 referents.					
Mackness	2003	UK	A follow-up study from Cherry et al 2002 (presenting more information on paraoxonase activity) 175 sheep dippers who reported illhealth.	234 sheep dippers who did not report ill health.	Serum and EDTA plasma were separated and stored at -20°C for no more than 3 months. Lithium-heparin plasma used immediately for liver function tests. PON1 activity and liver function not undertaken in 12 cases and 18 controls due to gross haemolysis.	Liver function Aspartate transaminase, Alanine transaminase, alkaline phosphatase, bilirubin and γ-glutamyltransferase. PON1 genotpyes identified from DNA extracted from lymphocytes. Serum PON1 activity towards paraoxon, phenylacetate and diazoxon. (For Paraoxonase 3 references to method, Davies HG Nature Genetics, 1996, 14, 334-336. Mackness B Br J Pharmacology, 1997, 112, 265-268. and La Du BN Fed proc, 1984, 43, 2338-2341.)	Not reported in this publication	Not reported	Students t-test for parameters with Gaussian distribution (age, PON1 activity towards diazoxon and phently acetate). Variables with non-gaussian distribution (PON1 activity towards paraoxon) were compared using Mann-Whitney U test. The chi-squared test was used to determine the significance of di
Povey A	2005	UK	A follow up study from Cherry 2002 and Mackness 2005 (presenting	Stepwise approach resulted in Full 234 referents	Subjects were sent health and exposure questionnaires to be completed in advance of a	PN1 55 and 192 genotypes reported	Not reported In this publication	Not reported.	A stepwise approach was used with subjects

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			an evaluation using a more stringent case definition) Based on original group of 409 subjects (ie combined case/referent group) Stepwise approach resulted in Full 175 cases Stage 1 169 Stage 2 161 Stage 3 142 Stage 4119 Stage 5 107	Stage 1 228 Stage 2 216 Stage 3 214 Stage 4 197 Stage 5 202	visit from a nurse who reviewed the questionnaire with the farmer, collected more exposure information, and took venous blood samples. Consent to approach GP obtained. GPs asked whether there was any history of neurological disease that could be confused with the effects of OP poisoning. Subjects were asked about health in previous month using an analogue scale to rate effects. There were 95 symptoms included. Subjects providing less than 90 responses were excluded.	No paraoxonase measurements..			(reporting answers for at least 90/95 symptom questions) with subjects excluded firstly on clinical grounds and then on the results of a discriminant analysis. Subjects excluded fell into four groups 1. Chronic condition attributed by GP to a clearly defined event (eg stroke), or causal agent (polio virus) or congenital condition (cerebral palsy) 2. Cases and referents with established neurological disease. 3. Subjects excluding those in group 1who were identified by discriminant analysis as having symptoms atypical of classification. 4. Subjects excluding those in groups 1 and

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									2 identified by discriminant analysis as having symptoms atypical of classification. Analyses in five stages Stage 1, all subjects excluding those with more than five unusable answers to symptom questions Stage 2all subjects except group 1 Stage 3 all subjects except groups 1 and 2. Stage 4 all subjects except group1 and group3 Stage 5 all subjects except groups 1,2 and 4.
Povey	2007b	UK	Study population was as reported in previous papers (Cherry 2002, Povey 2005, Cherry 2011)	Referent s as in previous studies.	Subjects were asked in detail regarding their health over the previous month, using a visual scale to rate 95 symptoms. Subjects who reported answers to at least 90 symptoms were included in the main analysis (mean response to symptoms assigned where five or fewer symptoms had been missed.	Genotyping for GSTM1*1/*2, GSTT1*1/*2 GSTP1*A/*B/*C, CYP2D6*3/*4, CYP3A4*1A/*1B CYP3A5*1/*3. CYP2D6*3/*4, was defined by identifying CYP2D6*3 and 2D6*4 alleles.	Every pesticide reported to have been used by each farmer for the period 1970-200 was extracted on to a database. All 1500 pesticides were decoded into their proper (trade) names, and given a unique code (580). Pesticides were		Frequencies were presented for categorical data and means/ SDs for continuous data. Ors and T-tests were used for categorical and continuous data respectively. Logisitc regression used

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
						CYPD2D6 wildtype was identified as having no *3 or *4 allele. CYP2D6 heterozygotes as having one *3 or *4 allele and CYP2D6 homozygotes as having two *3 or *4 alleles or one *3 or *4 allele. CYP2D6 HOMs were classified as poor metabolisers. Serum PON1 activity towards diazinon measured Davies HG Nature Genetics 14, 334-336, 1996.	categorised as Ops (and specifically diazinon) or non-OPs or could not be categorised.		to calculate ORs. Adjustment for gender, age at first dipping and number of years dipping had little effect on results and unadjusted data presented throughout. Initial analyses included all cases and referents recruited. Additional analyses were undertaken for populations known to be exposed to diazinon (255) For two exposure groups (392 without consideration of exposure and 255 exposed to diazinon) further refinement was undertaken using the exclusion of the approach outlined in Povey 2005
Cherry	2011	UK	Study population recruited as in Cherry 2002. originally 175 cases Information on possible dippers flu was expanded during nurse interview.	234 referents (referred to as no chronic illness or not chronic) Of these 42 reported that they had at some point experienced dippers flu.	Symptoms for each year from 1970-2000 collected. For each subject a report of dippers flu at any point in the interview was recorded and the date of first report extracted. No definition of dippers flu was offered and in the small number of	Relationship of dippers flu to exposure history. Relationship to PON1 polymorphisms and diazinon hydrolysis Interaction	Information on dipping practices Indices calculated Total years dipping Total number of sheep dipped Total number of times sheep dip	Not reported	Presence or absence of report of dippers flu related to each of the features of dipping with mean exposures compared by t-

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			(referred to in paper as chronic group). Of these 116 reported they had experienced dippers flu.		cases where a subject reported he did not know if the symptoms were dippers flu, the answer was considered positive if he reported symptom details.	between genotype and concentrate handling Case-crossover ananlysis. Survival analyisi (time taken from first dipping to onset of dippers flu) Paraoxonase methods as per Mackness B 2003 above)	concentrate handled Total years worked as plunger (dipping the sheep) Total years using diazinon Total years using dip believed to be an OP Ever handled diazinon concentrate For subjects who had started dipping after 1970 and who developed dippers flu in a year immediately following a dipping year with no symptoms, answers in each of the 2 years (one with dippers flu, the preceding one without) were extracted for more detailed case-crossover analysis of precipitating factors.		tests and presence or absence of susceptibility polymorphisms in PON1 by logistic regression. For case-cross over, students t-test to compare mean differences in exposure between the two years. Cox regression to analyse survival. To increase precision analyses were also restricted to those not reported by their family doctor to be suffering from one of a series of conditions with neurological symptoms (as reported in Povey 2005 (above)).
Manthripra-gada	2010	USA	Recruited between 1 Jan 2001-1 Jan 2008. 1167 cases invited, 563 were eligible (Parkinson's Disease (PD) diagnosis no longer than 3 years before recruitment, sufficiently healthy	Recruited between 1 Jan 2001-1 Jan 2008. Random sample from Medicare enrolees and from homes identified at random from tax assessor housing unit data listed on residential parcel maps. 1297	Assessment of PD status Genotyping using whole blood or buccal cell samples. For PON1 55	Assessment of PD status and polymorphism data for PON1 55	Residential exposure calculated from residential history and a GIS-based system which combined pesticide use reporting data and land use maps. A time specific average exposure was calculated for	Adjusted for sex, smoking, age, education (,12, 12, >12 years), county (Fresco, Tulare, Kern) and race (white, black, Latino, Asian, native American). In some analyses adjusted for	The Hardy-Weinberg equilibrium for PON1 55 in controls was assessed using chi-squared test. Compared genotype frequencies using a recessive

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			to be examined, currently residing in one of three counties, residing in california for at least 5 years) 473 eligible cases considered by UCLA movement disorder specialist who confirmed clinically probable or possible PD. 90 not interviewed (46 withdrew, 27 too ill or died, 17 moved out of area or did not attend). 96 examined but excluded due to other diagnoses other then PD. Of 377 cases remaining 9 did not provide all demographic data, not able to estimate pesticide exposure in 12, no DNA sample for 4, DNA sample failed during genotyping in one case. 351 cases included in assessment..	contacted. Eligibility included, not having PD, being at least 35 years, current residence in one of three counties of interest, residing in California for at least 5 years prior to recruitment, not being too ill.. One person per parcel unit allowed to enrol. Of 822 eligible controls, 414 did not participate (declined or moved from area prior to interview), 5 did not provide adequate demographic data, unable to estimate pesticide exposure for 15, no DNA sample for 24, and one sample failed during genotyping. There were 363 controls.			each subject. Pounds of pesticide applied per year within a 500m buffer of each residence and weighted this by the proportion of acreage treated within the buffer. The period specific average of all pesticide applications separately for diazinon, chlorpyrifos and parathion and the group of all other OPs (n=64) applied in California agriculture between 1974-1999. Occupational pesticide exposure estimates based on lifetime history of titles and self-reports of agricultural pesticide applications and information pertaining to tasks involving pesticides. Study participants were considered, not exposed, possibly exposed (grading, sorting, packing, office tasks in agricultural sector) or likely exposed (general	occupational exposure, and exposure to all other reported Ops (defined as greater than median exposure to 3 or more OPs excluding diazinon, chlorpyrifos and parathion between 1974-1999)	inheritance model comparing cases and controls carrying 2 variant alleles to those carrying one or more wild type alleles. This was chosen because the lowest activity has been reported for homozygous PON1 55 MM in human serum irrespective of PON1-108 and PON 192 variants, whereas paraoxonase activity among heterozygotes of PON1-55 varied with other PON variants. (O'Leary KA Pharmacogenetics Genomics, 2005, 15, 51-60. Median pesticide exposure used as a cutpoint for low/high exposure. Participants with missing residential pesticide exposure estimates were omitted (12 cases, 15 controls) (but

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
							farm work, ground maintenance, pesticide application)		were included in a sensitivity analysis. Assessed main effects of both genes and pesticides using an unconditional logistic regression analysis to calculate ORs (and 95%Cis). Joint effects estimates were calculated from combinations of alleles and pesticide exposure and entering these terms into logistic regression models. Multiplicative interaction was assessed by introducing an interaction term into the models. Stratified by sex, age at diagnosis (≤60, >60 years) For genetic and gene-environment analyses, sensitivity analyses were performed using only white persons.

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
6.B.3. Cross-sectional studies									
Lee	2003	USA	347 subjects (68% of eligible farm workers), from 75 participating farms (65% of eligible farms) 100 were selected by convenience had blood samples drawn towards the end of the study for PON1 genotyping. Of the 100 subjects selected 49 were pesticide applicators.	One non-applicator from same farms for every two applicators matched for age (2years) and educational status (1 year). Although not stated, these subjects were included in the 347 subjects. Of the 100 subjects selected 51 did not apply pesticides	Demographic information, symptoms, habits (pack years of smoking, alcohol use and problematic drinking), medical history, and self reported head injuries Symptoms had to be present within 3 months of the survey. Following symptoms were compatible with chronic toxicity (considered consistent with chronic OP toxicity) , chronic abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbances, limb numbness, paraesthesia, limb pain, limb weakness. Subject scores of a point for each symptom. ≥2 categorised as having toxic symptoms, ≤1 not having toxic symptoms. This divided the population in half.	Blood samples from study population for γ-glutamyl transferase, serum albumin and plasma and erythrocyte cholinesterase.	Study report states that farm workers were exposed to OPs, but no details were given.	Not reported	2-tailed t-test for unpaired data. Wilcoxon rank test for continuous normal variables, Fisher exact test for continuous non-normal variables. Mantel-Heanszel test for trend was used to compare pesticide-exposure status-genotype categories and frequency of toxic symptoms. Variables found to have a significant or near significant ($P<0.10$) association were included in the multiple logistic regression model.
Browne	2006	Israel	291 (149 male) 142 female (age range 4-90 years (mean 36.5 SD 21.7 years) from rural community where OP pesticides were regularly used. 28 excluded on basis of CNS related diseases. 60 gave written consent,	Controls taken from urban areas with no history of exposure to anticholinesterase s agents, no know neurological or psychiatric conditions. Biochemical data were available for 91 control. Data from same lab with	Blood samples used citrate as an anti coagulant. Plasma and whole blood samples stored at-70^{o}C until use. Genotyping for PON1 Plasma paraoxonase microtitre plates assay adapted from Furlong CE Anal Biochem, 180, 242-247, 1989. Using paraoxon	PON-1 Q192R, PON1 L55M polymorphisms. EEG data LORETA data	OP pesticides reported to be frequently used in the rural area (including fenitrothion, chlorpyrifos, monocrotophos, ethion and azinphos-methy)	Not reported	Analysis using Students t-test for AChE, arylesterase, paraoxonase. AUC for each discrete band in EEG. Statistically significant differences between LORETA values tested by using

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			with no history of acute intoxication. Blood samples for genotyping obtained from 30.	same techniques as present study. Characteristics of this group were not reported.	as substrate. Plasma arylesterase and cholinesterase also measured. Data from EEG measurements, and Low Resolution brain electromagnetic tomography (LORETA) undertaken. Methods as in Browne summary in neurophysiology section.				non-parametric t-test on a voxel by voxel basis with correction for repeated measure. (Three statistical thresholds were use P<0.01, P<0.05, P<0.1
6.B.4. Case series studies - none									
6.B.5. Case reports - none									

Table 7. POLYMORPHISM RESULTS AND CONCLUSIONS

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
B. Chronic Low-Level Exposure				
7.B.2. Case-control studies				
Cherry	2002	PON1 POLYMORPHISMS IN FARMERS ATTRIBUTING ILL HEALTH TO SHEEP DIP EXPSOURE: Time spent dipping was significantly less in cases compared to referents (19.4 (SD 7.5) years in cases, compared to 22.0 (7.2) years in referents). Two-thirds of all participants reported they had at some time used dips containing diazinon (115 (66%) cases, 152 (65%) referents. 88 (22%) of participants had not used diazinon but had used some organophosphate. 54 (13%) could not give sufficient details for classification of dip use. HEALTH OUTCOME; Linkage disequilibrium all people with RR (rarest polymorphism at 192), also had LL at position 55. (some characteristics of cases/controls reported in Mackness 2003 below) Cases were less likely than referents to be homzygous for QQ at position 192 (69 (39%) cases,	<u>Strengths</u> Genotyping of 409 sheep farmers who used dips. Diazoxonase activity measured. <u>Weaknesses</u> Case definition based on self reported illhealth. No specific investigation of chronic ill health	The authors concluded that PON 192 R allele polymorphism was associated with diminished ability to hydrolyse diazinoxon and was more frequent in people reporting ill health, as was LL polymorphism at position 55.

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
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140 (60%) referents and were more likely to be homozygous for LL at position 55 (86 (49%), 74 (32%) respectively. The frequency of the R allele was 0.35 in cases and 0.23 in controls (p<0.01). The frequency of the L allele was 0.71 in cases and 0.58 in controls (P<0.05) Odds ratios for polymorphisms are reported below
<table>
<tr><th></th><th>Odds ratio</th><th>p</th></tr>
<tr><td>Position 192</td><td></td><td>0.001</td></tr>
<tr><td>QR or RR vs QQ</td><td>2.25 (1.49-3,42)</td><td>0.001</td></tr>
<tr><td>RR vs QR or QQ</td><td>1.11 (0.50-2.44)</td><td>0.800</td></tr>
<tr><td>Position 55</td><td></td><td>0.001</td></tr>
<tr><td>LM or LL vs MM</td><td>1.55 (0.79-3.07)</td><td>0.204</td></tr>
<tr><td>LL vs LM or MM</td><td>1.92 (1.26-2.93)</td><td>0.002</td></tr>
<tr><td>Positions 192 and 55</td><td></td><td></td></tr>
<tr><td>QR or RR vs QQ</td><td>1.93 (1.24-3.01)</td><td>0.004</td></tr>
<tr><td>RR vs QR or QQ</td><td>0.84 (0.37-1.91)</td><td>0.674</td></tr>
<tr><td>LM or LL vs MM</td><td>1.21 (0.60-2.45)</td><td>0.599</td></tr>
<tr><td>LL vs LM or MM</td><td>1.70 (1.07-2.68)</td><td>0.024</td></tr>
</table>
Serum hydrolysis of diazinoxon was below the median of 14.7 µmol min^{-1} mL^{-1} in 58 % (95) of cases but only 44% (94) of referents. Hydrolytic activity was linked with polymorphisms at position 192 (participants below median; QQ 36% (67), QR 61% (97), RR 85% (23) p<0.0001.

At site 192 one allele of type R predisposed towards reported illhealth but possession of both alleles of this type was not an additional disadvantage. At site 55 additional risk of perceived illhealth was seen only when both alleles were of type L. This pattern was unchanged when both sites were analysed (overall effect at 192 p=0.015, 55 p=0.045). The interaction term was not significant (p=0.75)

The authors reported that the association of polymorphisms with ill health remained significant when subjects reporting no heart disease were removed (137 cases, 214 referents) OR at 192= 1.79 (95%CI 1.13-2.84), 55= 1.93 (95% CI 1.21-3.08)

The unadjusted OR for diazinoxon activity below median was 1.77 (1.12-2.67). The adjusted ORs including both polymorphisms and activity were
Position 192=1.49 (95% CI 0.94-2.37)
Position 55= 1.90 (95%CI 1.19-3.03)
Activity 1.76 (95% CI 1.12-2.76)
</td>
<td></td>
<td>Cases were more likely to have low serum hydrolytic activity for diazinoxon. The results supported the hypothesis that organophosphates contribute to reported ill health of people who sheep dip</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Mackness B</td><td>2003</td><td>PON1 POLYMORPHISMS AND PARAOXONASE ACTIVITY IN FARMERS ATTRIBUTING ILL HEALTH TO SHEEP DIP

EXPOSURE; Not specifically measured in this study.

HEALTH OUTCOMES: Cases were reported to be very similar to controls. Thus 89.1% of cases and 89.7% of controls were male. Mean age at interview was 53.9 ±10.4 years for cases and 51.8±11.5 year for controls. Total time spent dipping was significantly less in cases compared to controls. The symptoms which best differentiated cases from controls were difficulty in concentrating and muscle spasm

The distribution of PON1 polymorphisms was as reported in Cherry et al 2002.

Serum activities towards paraoxon, diazoxon and phenyl-acetate are shown below. There were no significant difference between cases/controls.

<table><tr><td></td><td>Controls</td><td>Cases</td></tr><tr><td>Number (M/F)</td><td>216 (194/22)</td><td>163 (145/18)</td></tr><tr><td>Age</td><td>53.9±10.4</td><td>51.8±11.5</td></tr><tr><td>Paraoxon*</td><td>146.8 (28.5-545.1)</td><td>155.6 (27.9-487.1)</td></tr><tr><td>Diazoxon**</td><td>15.0±5.3</td><td>14.1±5.3</td></tr><tr><td>Phenyl-acetate**</td><td>78.4±23.9</td><td>78.0±23.4</td></tr></table>* nmol/min/ml serum
** µmol/min/ml serum

However when the two groups were divided into quintiles according to diazoxon hydrolysis, farmers in the lowest quintile had a greater risk of being a case (OR 2.47 (95%CI 1.23-2.82) than those in the other 4 quintiles. There were no difference in Ors for paraoxon and phenyl-acetate.

In both cases and controls possession of the Q allele increased the rate of diazoxon hydrolysis (QQ had highest activity, QR intermediate and RR homozygotes the lowest activity) the pattern was reversed for paraoxon. There was also a trend similar to paraoxon for phenylacetate.</td><td><u>Strengths</u>
Genotyping of 409 sheep farmers who used dips. Data on paraoxonase activity to several substrates.
<u>Weaknesses</u> Case definition based on self reported illhealth. No specific investigation of chronic ill health</td><td>The authors concluded that subjects with the lowest quintile of diazoxon hydrolysis were 2.5x more likely to report ill health. Farmers reporting ill health more commonly had R and L PON1 alleles. R/L haplotype was associated with lower diazinoxon hydrolysis (not significant in cases).</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
PON1 192
<table>
<tr><td></td><td>Controlls QQ</td><td>Controls QR</td><td>Controls RR</td><td>Cases QQ</td><td>Cases QR</td><td>Cases RR</td></tr>
<tr><td>n</td><td>127</td><td>77</td><td>12</td><td>65</td><td>83</td><td>15</td></tr>
<tr><td>Paraoxon* (median and range)</td><td>104.1 (28.5-216.7)</td><td>231.4 (58.9-406.5)</td><td>403.6 (183.7-545.1)!</td><td>83.6 (27.9-211.4)</td><td>215.5 (35.1-443.6)</td><td>244.4 (103.9-487.0) !***</td></tr>
<tr><td>Diazoxon**</td><td>16.6 ±5.5</td><td>13.1±4.1</td><td>10.8±3.3!</td><td>15.8±6.5</td><td>13.6±3.8</td><td>9.6±2.5!</td></tr>
<tr><td>Phenylacetate**</td><td>74.3±19.0</td><td>83.9±28.9</td><td>89.3± 26.4! !</td><td>75.9±19.7</td><td>77.4±22.4</td><td>89.7±38.3</td></tr>
</table>
* nmol/min/ml serum
** µmol/min/ml serum
! Significantly different by ANOVA P<0.0001
! !P=0.0054
*** Significantly different from controls P=0.0081

The effects of PON1-55 polymorphism on paraoxon hydrolysis for the L showed that LL homozygotes had the highest activity, LM heterozygotes were intermediate, and MM homozygotes had the lowest activity. The L allele was also associated with higher rates of serum diazoxon hydrolysis in cases. This pattern was less evident in controls (not statistically significant). Diazoxon hydrolysis was significantly (P<0.05) lower in LL homozygotes, intermediate in LM and lowest in MM homozygotes.

PON55
<table>
<tr><td></td><td>Controlls QQ</td><td>Controls QR</td><td>Controls RR</td><td>Cases QQ</td><td>Cases QR</td><td>Cases RR</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>n</td><td>68</td><td>116</td><td>32</td><td>79</td><td>70</td><td>14</td></tr>
<tr><td>Paraoxon* (median and range)</td><td>179.0 (32.3-545.1)</td><td>142.3 (36.2-406.5)</td><td>80.0 (28.5-308.8)!</td><td>194.7 (35.1-487.1)</td><td>137.2 (35.8-443.6)</td><td>61.6 (27.9-189.5)!</td></tr>
<tr><td>Diazoxon**</td><td>16.4±6.4</td><td>14.4±4.8</td><td>14.3±3.7</td><td>15.0±6.3</td><td>13.6±4.0</td><td>11.8±3.8 !! ***</td></tr>
<tr><td>Phenylacetate**</td><td>83.8±20.6</td><td>77.4±26.1</td><td>71.3±20.3 !!</td><td>83.3±23.3</td><td>74.0±22.0</td><td>68.2±25.4!!</td></tr>
</table>
* nmol/min/ml serum
** µmol/min/ml serum
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
! Significantly different by ANOVA P<0.0001
! !P<0.05 or 0.02
*** Significantly different from controls P<0.05

In combined genotype groups paraoxon hydrolysis was significantly lower in RL haplotype (P<0.01). Diazoxon hydrolysis was lower in RR/LL genotype compared to other genotypes (P<0.001) Multiple regression analysis indicated that PON1-55 and 192 polymorphisms independently affected diazoxon hydrolysis in both cases and controls. (both P<0.0001)

<table>
<tr><th>PON1 55/192</th><th>n Cases:controls</th><th>Diazinoxon controls</th><th>Diazinoxon Cases</th><th>Paraoxon Controls</th><th>Paraoxon Cases</th></tr>
<tr><td>QQ/MM</td><td>31:13</td><td>14.3±3.7</td><td>11.9±3.9</td><td>77.8(28.5-159.3)</td><td>57.1 (27.9-189.5)</td></tr>
<tr><td>QQ/LM</td><td>70:32</td><td>16.2±4.8</td><td>14.9±4.5</td><td>105.0 (36.2-216.7)</td><td>90.1 (35.2-204.8)</td></tr>
<tr><td>QQ/LL</td><td>26:19</td><td>20.1±7.2</td><td>20.3±8.4</td><td>160.7 (32.3-215.6)</td><td>89.8 (40.1-211.4)</td></tr>
<tr><td>QR/LM</td><td>46:36</td><td>11.6±3.4</td><td>12.4±3.2</td><td>255.3 (58.9-406.5)</td><td>237.2(84.1-443.6)</td></tr>
<tr><td>QR/MM</td><td>1:1</td><td>14.5</td><td>10.1</td><td>308.8</td><td>140.5</td></tr>
<tr><td>QR/LL</td><td>30:47</td><td>15.3±4.1</td><td>14.5±4.0</td><td>197.8 (84.1-374.9)</td><td>200.1 (35.1-383.4)</td></tr>
<tr><td>RR/LL</td><td>12:15</td><td>10.7±3.3*</td><td>9.6±2.5*</td><td>403.6 (183.7-545.1)</td><td>244.4 (103.9-487.1)!</td></tr>
</table>
*Significantly different from other genotype combinations P<0.001
! Significantly different from controls P<0.01
No individuals with RR/MM or RR/LM combinations were identified.
Diazinoxon hydrolysis µmol/min/ml
Paraoxon hydrolysis nmol/min/ml
</td><td></td><td></td></tr>
<tr><td>Povey</td><td>2005</td><td>PON1 POLYMORPHISMS IN FARMERS ATTRIBUTING ILL HEALTH TO SHEEP DIP: FURTHER ANALYSES USING STEPWISE APPROACH TO EXCLUDE SUBJECTS BASED ON CLINICAL FEATURES AND DISCRIMINANT ANALYSIS

EXPOSURE: Not reported in this study.</td><td><u>Strengths</u>
Genotyping of 409 sheep farmers who used dips. Improved classification of</td><td>Authors concluded that risk associated with PON1-192 or-55 genotypes did not</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
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<td>
HEALTH OUTCOMES: 409 subjects in initial analyses, of which 12 gave responses to less than 90 symptoms and were excluded. Information from GPs for 402 subjects.
Cases were more likely than referents they had been troubled by ill health during the past month and more likely than referents to report they felt their health had been affected by sheep dipping.

A total of 20 subjects (eight cases) were excluded on basis of clinical grounds, 7 stroke, 13 trauma.
A total of 21 subjects had established neurological disease (19 cases). This included 7 cases of multiple sclerosis, 2 of Alzheimer's and 2 of Parkinson's disease.

After exclusion of group 1, discriminant analysis correctly identified 83.8% of cases. A total of 61 subjects (42 cases) were identified as belonging to group 3 (excluding those in group 1 subjects with atypical symptoms).

After excluding groups 1 and 2 (41 subjects), discriminant analysis correctly identified 86.6% of cases. A total of 47 subjects (35 cases) were identified as belonging to group 4 (excluding those in group 1,2 subjects with atypical symptoms.

The risk associated with QR or RR remained elevated (>2 fold) when compared to QQ after exclusion of subjects on clinical grounds or by discriminant analysis. Risk of QR remained similar but risk of RR (vs QQ) was reduced in subjects excluded by discriminant analysis. The risk associated with LL genotype remained elevated when compared to LM or MM genotype even after subject exclusion. The decreased risk associated with MM was lower than LM genotype.

PON1- 192
<table>
<tr><th>Analysis stage</th><th>Total N</th><th>OR (95%CI) QR or RR vs QQ</th></tr>
<tr><td>Full</td><td>409</td><td>2.25 (1.49-3.42)</td></tr>
<tr><td>Stage 1</td><td>397</td><td>2.29 (1.53-3.44)</td></tr>
<tr><td>Stage 2</td><td>377</td><td>2.25 (1.48-3.44)</td></tr>
<tr><td>Stage 3</td><td>356</td><td>2.50 (1.62-3.87)</td></tr>
<tr><td>Stage 4</td><td>316</td><td>2.08 (1.31-3.30)</td></tr>
<tr><td>Stage 5</td><td>309</td><td>2.45 (1.51-3.97)</td></tr>
</table>
</td>
<td>cases and referents to exclude subjects with neurological conditions and symptom patterns atypical of exposure.
Weaknesses Case definition based on self-reported symptoms. No specific investigation of chronic ill health. Exposure-response not investigated. Paraoxonase activity not investigated.</td>
<td>alter significantly when the population studied was refined to give a more homogeneous study group.</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>PON1-55
<table>
<tr><th>Analysis stage</th><th>Total N</th><th>OR (95%CI) LL vs LM or MM</th></tr>
<tr><td>Full</td><td>409</td><td>1.92 (1.26-2.93)</td></tr>
<tr><td>Stage 1</td><td>397</td><td>2.10 (1.39-3.16)</td></tr>
<tr><td>Stage 2</td><td>377</td><td>2.10(1.38-3.21)</td></tr>
<tr><td>Stage 3</td><td>356</td><td>2.15 (1.39-3.32)</td></tr>
<tr><td>Stage 4</td><td>316</td><td>1.79 (1.12-2.85)</td></tr>
<tr><td>Stage 5</td><td>309</td><td>1.95 (1.21-3.15)</td></tr>
</table></td><td></td><td></td></tr>
<tr><td>Povey</td><td>2007b</td><td>POLMORPHISMS OF CYP ENZYMES AND PON1 IN SHEEP DIPPERS

EXPOSURE: Exposure data were not separately reported in this paper.

HEALTH OUTCOMES: The study population was as reported in Cherry 2002 and Mackness et al 2003).

<u>PON1 genotype and phenotype</u>.

There was a significant difference in frequency of PON1-55 and PON1-192 genotypes in cases and referents. Both in whole study population and referents with some diazinon exposure. There was an elevated risk associated with having low diazoxonase which was not greater in the diazinon-exposed population than all subjects.
<table>
<tr><th>Variable</th><th>Exposure (n)</th><th>Cases</th><th>Referents</th><th>OR</th></tr>
<tr><td>PON1-55 LL/LM/MM (%LL)</td><td>All (409)</td><td>86/75/14 (49.1%)</td><td>74/124/36 (31.6)</td><td>1.92 (1.26-2.93)**</td></tr>
<tr><td>PON1-55 LL/LM/MM (%LL)</td><td>Diazinon (267)</td><td>59/46/10 (51.3%)</td><td>38/89/25 (25.0)</td><td>3.16 (1.88-5.31)**</td></tr>
<tr><td>PON1-192 QQ/QR/RR (%QQ)</td><td>All (409)</td><td>69/90/16 (39.4%)</td><td>140/81/13 (59.8)</td><td>2.25 (1.49-3.42)***</td></tr>
<tr><td>PON1-192 QQ/QR/RR</td><td>Diazinon (267)</td><td>48/53/14 (41.7)</td><td>96/48/8 (63.2)</td><td>2.39 (1.45-3.93)***</td></tr>
</table></td><td>Strengths
Genotyping data for a range of genotypes other than PON1 in cases with self-reported OP chronic toxicity compared to referents. Diazonoxonase activity measured in subjects. A selection process was used to exclude subjects with neurological conditions and with symptoms un-related to OP exposure,

Limitations Relied on self-reports of chronic illhealth.</td><td>Overall there was some evidence for an association between risk of chronic ill health and genes of OP metabolism but the results were either not significant or not consistent with the apriori hypothesis that faster metabolic activation by CYP2D6 WT should be associated with elevated risk.

Authors report other published</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><td>(%QQ)</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Diazoxon hydrolysis µmol/min/ml mean ±SD</td><td>All (379)</td><td>14.1 ±5.3</td><td>15.0±5.3</td><td>P = 0.10</td></tr>
<tr><td>Diazoxon hydrolysis µmol/min/ml mean ±SD</td><td>Diazinon (247)</td><td>14.2±5.7</td><td>15.2±4.8</td><td>P=0.12</td></tr>
<tr><td>Diazoxon hydrolysis Low/High activity (%low)</td><td>All (379)</td><td>95/69 (57.9)</td><td>94/121 (43.7)</td><td>1.77 (1.15-2.73)****</td></tr>
<tr><td>Diazoxon hydrolysis Low/High activity* (%low)</td><td>Diazinon (247)</td><td>63/45 (58.3)</td><td>59/80 (42.5)</td><td>1.90 (1.11-3.27)****</td></tr>
</table>
* based on median diaoxonase activity (14.2 µmol/min/ml)
** LL vs LM/MM
***QR+RR vs QQ
**** Low vs high activity

<u>GST genotype</u>

There were no significant differences in the frequency of GSTM1*2 (null) genotype and GSTT1*2 (null) genotype in cases and referents in either whole population or in only those who were exposed to diazinon. Restricting the number of subjects by applying the stages of the discriminant analysis did not affect these results.

In the whole population there was an elevated but non-significant risk associated with combined GSTM1 and GSTT1 null genotype (9% of cases, 5.8% of referents were double-null homozygotes. (OR= 1.61 (95%CI 0.74-3.48)). In the staged analysis there was evidence of an increased risk after exclusion of subjects on clinical grounds. 10.4 % of cases were double null homozygotes at stage 3 compared to 5.4% of referents. (OR 2.06 (0.85-5.04)). A similar result was reported in restricted analysis of subjects exposed to diazoxon (OR = 2.60 (0.72-10.42))
</td>
<td>Phenotype for CYP and GSTP not measured. Some results did not accord with a priori hypothesis in that associations were found with CYP genotypes associated with slower metabolism of OPs.</td>
<td>papers which relate to PON1 and pesticide exposure, presumably not OP specific which haven't been captured in the current literature review (Hernandez Hum Exp Toxicol, 22, 505-574, 2003)</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Analysis stage*</td><td>GSTM1/GSTT1 null (All)</td><td>OR (95% CI)</td><td>GSTM1/GSTT1 null (Diazinon)</td><td>OR (95% CI)</td></tr>
<tr><td>Full</td><td>392</td><td>1.61 (0.70-3.71)</td><td>255</td><td>1.76 (0.58-5.44)</td></tr>
<tr><td>1</td><td>380</td><td>1.77 (0.76-4.17)</td><td>247</td><td>2.05 (0.63-7.23)</td></tr>
<tr><td>2</td><td>360</td><td>1.94 (0.81-4.67)</td><td>236</td><td>2.41 (0.69-9.41)</td></tr>
<tr><td>3</td><td>339</td><td>2.06 (0.85-5.04</td><td>215</td><td>2.60 (0.72-10.42)</td></tr>
<tr><td>4</td><td>301</td><td>1.91 (0.76-4.85)</td><td>191</td><td>2.23 (0.58-9.25)</td></tr>
<tr><td>5</td><td>293</td><td>2.26 (0.89-5.75)</td><td>186</td><td>2.73 (0.71-11.33)</td></tr>
</table>

Stepwise approach summarised below for ease of reference.
{A stepwise approach was used with subjects (reporting answers for at least 90/95 symptom questions) with subjects excluded firstly on clinical grounds and then on the results of a discriminant analysis. Subjects excluded fell into four groups
1. Chronic condition attributed by GP to a clearly defined event (eg stroke), or causal agent (polio virus) or congenital condition (cerebral palsy)
2. Cases and referents with established neurological disease.
3. Subjects excluding those in group 1who were identified by discriminant analysis as having symptoms atypical of classification.
4. Subjects excluding those in groups 1 and 2 identified by discriminant analysis as having symptoms atypical of classification.
Stage 5 all subjects except groups 1,2 and 4.

Analyses in five stages
Stage 1, all subjects excluding those with more than five unusable answers to symptom questions
Stage 2all subjects except group 1
Stage 3 all subjects except groups 1 and 2.
Stage 4 all subjects except group1 and group3
Stage 5 all subjects except groups 1,2 and 4.}

With regard to GSTP1, the risk of having a *B or *C allele was slightly increased in the whole study population and in those exposed to diazinon but none of the reported ORs reached significance.
<table>
<tr><td>Analysis stage</td><td>GSTP1* null (all subjects)</td><td>OR (95%CI)*</td><td>GSTP1* null (diazinon exposed)</td><td>OR (95%CI)</td></tr>
<tr><td>Full</td><td>383</td><td>1.33 (0.88-2.01)</td><td>247</td><td>1.39 (0.63-3.09)</td></tr>
<tr><td>1</td><td>372</td><td>1.31 (0.84-2.05)</td><td>240</td><td>1.43 (0.82-2.51)</td></tr>
</table>
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>2</td><td>353</td><td>1.40 (0.88-2.22)</td><td>230</td><td>1.66 (0.94-2.96)</td></tr>
<tr><td>3</td><td>333</td><td>1.27 (0.79-2.05)</td><td>210</td><td>1.44 (0.78-2.64)</td></tr>
<tr><td>4</td><td>296</td><td>1.28 (0.76-2.15)</td><td>187</td><td>1.88 (0.96-3.67)</td></tr>
<tr><td>5</td><td>289</td><td>1.24 (0.73-2.12)</td><td>183</td><td>1.82 (0.92-3.63)</td></tr>
</table>
GSTP1 *A*B+ GSTP1 *A*C+ GSTP1 *B*B+ GSTP1 *B*C+ GSTP1 *C*C vs GSTP1*A*A

<u>CYP genotype</u>

The risk associated with CYP2D6 wild type (defined using CYP2D6*3 and CYP2D6*4) was elevated but not significantly (OR 1.47 (95%CI 0.83-2.60)) Restricting the population using the staged assessment did not alter the results. There were no significant differences in the distribution of CYP3A4 and 3A5 genotypes between cases and referents. Subjects homozygous for CYP3A4*1B or CYP3A5*3 were rare with only one of each identified in this population. The CYP3A4*A/*B allele frequency did not differ between cases and referents (0.975/0.025 and 0.964/0.036 respectively). CYP3A5*1*3 allele frequency did not differ between cases (0.957/0.043) and referents (0.942/0.057).

<u>Combined PON1, GST and CYP genotypes</u>

The OR (95%CI) for PON1 QR/RR polymorphisms was 5.75 (1.22-29.11) in subjects characterised as either CYPD2D6 HET or CYPD2D6 HOM and 1.81 (0.99-3.38) in CYPD2D6 WT subjects. For PON1 LL the OR (95%CI) was 3.98 (2.08) for CYPD2D6 WT and 1.11 (0.23-4.98) for CYPD2D6 HET/HOM. For GSTP1 there was an increased OR for all combinations except *A*A (see table below). There were no increased ORs for PON1 polymorphisms combined with GSTT1 or GSTM1 except for and association between PON1 LL with GSTT1*1 (4.08 (2.15-7.76) (OR for GSTT1*2 was1.21 (0.29-5.16)).

<table>
<tr><th>Variable</th><th>Comparison</th><th>CYPD2D6 WT</th><th>CYPD2D6 HOM/HET</th><th>GSTP1 A*A</th><th>GSTP1 all other combinations.</th></tr>
<tr><td>PON1-55</td><td>All exposure LL vs LM/MM</td><td>2.18 (1.35-3.47)</td><td>1.39 (0.47-4.11)</td><td>2.26 (1.09-4.66)</td><td>4.31 (1.69-11.17)</td></tr>
<tr><td>PON1-55</td><td>Diazinon exposure LL vs LM/MM</td><td>3.98 (2.08-7.68)</td><td>1.11 (0.23-4.98)</td><td>1.63 (0.90-2.96)</td><td>2.27 (1.06-4.87)</td></tr>
<tr><td>PON1-192</td><td>All exposure QR/RR vs</td><td>1.93 (1.23-3.02)</td><td>3.30 (1.08-10.07)</td><td>1.37 (0.68-2.76)</td><td>2.22 (1.23-4.02)</td></tr>
</table>
</td><td></td><td></td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions

	QQ				
PON1-192	Diazinon exposure QR/RR vs QQ	1.81 (0.99-3.08)	5.75 (1.72-29.11)	1.80 (1.20-6.67)	2.68 (1.29-5.60)
Diazoxonase activity	All exposure Low v High	1.63 (1.00)	2.48 (0.69-9.07)	1.16 (0.56-2.40)	2.05 (12.14-3.70)
Diazoxonase activity	Diazinon exposure Low v High	1.67 (0.90-3.10)	5.70 (1.03-36.84)	0.99 (0.40-2.40)	2.76 (1.28-5.98)

Combined diazoxonase activity, CYP, GST genotypes

Risk associated with low diazoxonase activity was higher in subjects genotypes as CYP2D6 HET or CYP2D6 WT. In those exposed to diazinon the risk associated with low diazoxonase activity was 5.50 (1.03-36.84) in subjects characterised as either CYPD2D6 HET or CYPD2D6 HOM but only 1.67 (0.90-3.10) in CYPD2D6 WT individuals.

There was little evidence that risk associated with low diazinoxonase activity varied with GSTT1 or GSTM1 genotype.

The risk of low diazinoxonase activity was higher in subjects with GSTP1 *B or *C alleles than GSTP1*A homozygotes. In those exposed to diazinon the OR (95%CI) associated with low diazinoxonase activity was 2.76 (1.28-5.98) in subjects with GSTP1 *B or *C alleles but 0.99 ().40-2.40) in GSTP1*A homozygotes.

The authors hypothesised that risks associated with CYP2D6 WT would be greater than CYP2D6 HET/HOM. The risk was increased in PON1-55 LL subjects with CYP2D6 WT. However increased risk associated with low diazinoxonase activity and PON1-192 QR/RR genotype was observed in individuals with lower CYP activity (CYP2D6 HET/HOM). These results were not consistent with the original hypothesis. The authors felt the results could be due to misclassification of the CYP 2D6 genotype or by chance.

There was some evidence for an association between PON1-55 (LL) and PON1-192 (QR/RR) and GSTP *B and GSTP*C polymorphisisms. If true metabolism of OPs would be different *in vivo* to that afforded by the GSTP *A allele. The authors suggest this hypothesis could be tested *in vitro*

The available evidence suggested that GSTM1 and GSTT1 genotypes were not involved in metabolism of OPs.

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Cherry</td><td>2011</td><td>DIPPERS FLU AND ITS RELATIONSHIP TO PON1 POLYMORPHISMS

EXPOSURE: Exposures were highly variable. Mean total number of sheep dipped since 1970 ranged from 24-4,000,000 with the highest numbers found for professional dippers who moved from farm to farm. The great majority (81.6%) had handled concentrates but the number of times ranged from twice to 33 060 (mean 1287). Pesticide use was not know in over 70% of subjects for at least 1 year. Where it was known, the vast majority of dips were OP based, predominantly diazinon. 28.1% reported using a non-OP dip, largely in early years before Op dips became common.

HEATH OUTCOMES: Those reporting ill health (considered as chronically unwell) and those not reporting ill health (no chronic illness)

<u>Relationship of dippers flu to exposure history</u>.
In the total group of chronically ill farmers, and also those who started after 1969, none of the indices of sheep dip were related to the presence of ever/never of dippers flu. In those without chronic ill health, the cumulative number of times pouring a concentrate was greater in those reporting dippers flu. In a further analysis comparing those ever handling with those never handling concentrate, in those who started dipping after 1969, an association with dippers flu was reported.

All subjects (results)
<table><tr><th>Health/exposure</th><th>Dippers Flu Mean (SD)</th><th>N</th><th>No Dippers flu (mean (SD)</th><th>N</th><th>P value</th></tr><tr><td>Chronically unwell</td><td></td><td></td><td></td><td></td><td></td></tr><tr><td>Total years dipped</td><td>20.1 (7.7|)</td><td>116</td><td>19.2 (7.5)</td><td>59</td><td>0.44</td></tr><tr><td>Total sheep dipped (x1000)</td><td>77.3 (192.6)</td><td>115</td><td>60.2 (141.8)</td><td>59</td><td>0.55</td></tr></table></td><td><u>Strengths</u>
Polymorphism data from sheep farmers with detailed exposure history. Questionnaire data on presence/absence of dippers flu

<u>Weaknesses</u>
Authors report case-referent recruitment was not ideal; referent group (Not chronic illness) represent ‘survivors’, Information on exposure was collected retrospectively over 30 years. Clear minded-dispassionate recall would have been difficult for those reporting chronic illness. Exposure to other environmental factors not reported. Exposure misclassification was possible.</td><td>Authors conclude that dippers flu and chronic ill-health attributed to dipping share a common polymorphism (R allele of PON1 192). The interaction between handling concentrate and PON1 genotype supports the conclusion that OPs may cause dippers flu.</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
<table>
<tr><td>Total concentrate handling</td><td>860.5 (3028.3)</td><td>115</td><td>932.3 (3286.7)</td><td>59</td><td>0.89</td></tr>
<tr><td>Total years as plunger</td><td>16.7 (9.6)</td><td>116</td><td>15.8 (9.8)</td><td>59</td><td>0.55</td></tr>
<tr><td>Total years diazinon</td><td>5.6 (6.8)</td><td>116</td><td>5.4 (7.0)</td><td>59</td><td>0.87</td></tr>
<tr><td>Total years any OP</td><td>10.1 (7.2)</td><td>116</td><td>9.8 (7.9)</td><td>59</td><td>0.79</td></tr>
<tr><td>No Chronic Illness</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Total years dipped</td><td>22.1 (6.5)</td><td>42</td><td>20.0 (7.3)</td><td>192</td><td>0.93</td></tr>
<tr><td>Total sheep dipped</td><td>241.9 (571.9)</td><td>42</td><td>133.8 (458.5)</td><td>192</td><td>0.19</td></tr>
<tr><td>Total concentrate handling</td><td>3194.2 (7355.1)</td><td>42</td><td>1220.7 (3727.5)</td><td>192</td><td>0.01</td></tr>
<tr><td>Total years as plunger</td><td>19.2 (9.2)</td><td>42</td><td>18.7 (9.9)</td><td>192</td><td>0.77</td></tr>
<tr><td>Total years diazinon</td><td>6.9 (7.6)</td><td>42</td><td>5.8 (7.6)</td><td>192</td><td>0.39</td></tr>
<tr><td>Total years any OP</td><td>12.5 (8.4)</td><td>42</td><td>10,8 (8.2)</td><td>192</td><td>0.23</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

Subjects with start date after 1970 had very similar results except the difference between those with dippers flu and those without dippers flu assessed for total concentrate handling was not significant. (2213.2 (6032.9) with dippers flu and 1422.3 (4712.1) without dippers flu p value 0.50. In a further assessment those ever handling concentrate compared with those never handling concentrate in the non chronic group who started dipping after 1969 were more likely to have dippers flu if they had ever handled concentrate (concentrate dippers flu (21/89, 23.6%) verses no concentrate (2/28, 7.1%) p=0.04.

<u>Relationship to PON1 polymorphisms and diazinon hydrolysis</u>
The occurrence of LL at position 55 was not related to dippers flu in any of the four study groups (All with chronic illness, all with chronic illness stating dipping after 1970, all with no chronic illness, and those without chronic illness who stated dipping after 1970)
</td>
<td>Authors noted weaker genotype association in not chronic group for total exposure compared to after 1970 exposure and considered possible selection bias excluding those susceptible among those who started dipping many years ago.

Paraoxonase activity not measured.</td>
<td></td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
The presence of one or more R alleles was associated with dippers flu in all four groups.

In chronic illness group those with two R alleles were at more risk than those with only one R allele. In those without chronic illness the same pattern of results were found but the number of subjects with RR was small.

Analysis of diazoxon hydrolysis was lower in those with R allele. Mean diazoxon hydrolysis was lower in those with dippers flu (not significant).

PON1 genotype and dippers flu (DF) PON1 192

<table>
<tr><td>Group</td><td>DF (QQ/QR/RR)</td><td>No DF (QQ/QR/RR)</td><td>OR (95% CI)</td></tr>
<tr><td>All chronic</td><td>39/68/8</td><td>30/21/8</td><td>2.04 (1.08-3.87)</td></tr>
<tr><td>Chronic ≥1970</td><td>18/33/2</td><td>10/9/5</td><td>1.39 (0.52-3.74)</td></tr>
<tr><td>All not chronic</td><td>23/15/4</td><td>117/66/9</td><td>1.29 (0.66-6.37)</td></tr>
<tr><td>Not chronic ≥1970</td><td>10/11/2</td><td>62/28/4</td><td>2.52 (1.00-6.37)</td></tr>
</table>

PON1 genotype and dippers flu (DF) PON1 55

<table>
<tr><td>Group</td><td>DF (LL/LM/MM)</td><td>No DF (LL/LM/MM)</td><td>OR (95% CI)</td></tr>
<tr><td>All chronic</td><td>57/49/10</td><td>29/26/4</td><td>1.00 (0.53-1.87)</td></tr>
<tr><td>Chronic ≥1970</td><td>25/23/5</td><td>13/9/2</td><td>0.76 (0.29-1.99)</td></tr>
<tr><td>All not chronic</td><td>11/24/7</td><td>63/100/29</td><td>0.73 (0.34-1.54)</td></tr>
<tr><td>Not chronic ≥1970</td><td>8/10/5</td><td>31/49/14</td><td>1.08 (0.42-2.83)</td></tr>
</table>

<u>Interaction between genotype and concentrate handling</u>

Exposure was redefined to include only those who had handled diazinon concentrate (pouring concentrate or adding it to the dipping bath) compared to all other subjects. Additionally subjects reported by family doctors to have neurological deficits (n=20) or diagnosed illnesses with neurological symptoms (n=22) were excluded.

In univariate analysis, the OR for the interaction is greater than that of either of the main effects in each study group suggesting those who handled concentrate and had an R allele were at greater risk of developing dippers flu. In the multivariable analysis with both main effects and interaction in the model, this size of interaction effect was greater than in the univariate analyses in those who were chronically ill. The authors suggest the risk of dippers flu was largely confined to those who handled concentrate and had the susceptibility allele. The multivariable analyses of those who were healthy in the original study showed a reduction of size of the interaction term
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
when both main effects were included in the model; the estimated risk associated with interaction was still >1.00 but might be due to chance.

Chronically Unwell

<table>
<tr><td>Assessment</td><td>Chronically unwell ALL Univariate OR (95%CI)</td><td>Chronically unwell ALL Multivariate OR (95%CI)</td><td>Chronically unwell ≥1970 Univariate OR (95%CI)</td><td>Chronically unwell ≥1970 Multivariate OR (95%CI)</td></tr>
<tr><td>Handled diazinon concentrate</td><td>1.31 (0.63-2.71)</td><td>0.65 (0.21-1.96)</td><td>1.06 (0.34-3.33)</td><td>0.23 (0.04-1.50)</td></tr>
<tr><td>Genotype at R position</td><td>2.20(0.97-4.21)</td><td>1.14 (0.42-3.08)</td><td>1.65 (0.55-4.91)</td><td>0.73 (0.18-2.94)</td></tr>
<tr><td>Interaction concentrate x genotype</td><td>3.00 (1.16-7.79)</td><td>3.87 (0.84-17.89)</td><td>3.20 (0.65-15.78)</td><td>12.77 (1.02-159.49)</td></tr>
</table>

<u>No Chronic illness</u>

<table>
<tr><td>Assessment</td><td>No chronic illness ALL Univariate OR (95%CI)</td><td>No chronic illness ALL Multivariate OR (95%CI)</td><td>No chronic illness ≥1970 Univariate OR (95%CI)</td><td>No chronic illness ≥1970 Multivariate OR (95%CI)</td></tr>
<tr><td>Handled diazinon concentrate</td><td>1.40 (0.70-2.78)</td><td>1.13 (0.47-2.89)</td><td>2.83 (1.08-7.45)</td><td>2.32 (0.56-9.58)</td></tr>
<tr><td>Genotype at R position</td><td>1.42 (0.71-2.83)</td><td>1.13 (0.42-3.00)</td><td>2.99 (1.15-7.79)</td><td>2.44 (0.54-10.96)</td></tr>
<tr><td>Interaction concentrate x genotype</td><td>1.82 (0.82-4.04)</td><td>1.54 (0.38-6.20)</td><td>4.05 (1.44-11.38)</td><td>1.19 (0.16-8.67)</td></tr>
</table>

<u>Case-crossover</u>

Information was obtained from subjects with exposure information for 2 years, specifically one year with the onset of dippers flu and the preceding one without. Dippers flu.
</td><td></td><td></td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		43 subjects met the criterion for case-crossover analysis, 31 from the chronic group and 12 from the group with no chronic illness. Of these 23 reported the number of sheep, number of days, number of times handling concentrate and type of dip were all identical in the year in which they developed dippers flu and the preceding year in which they did not report dippers flu. In the remaining 23, the mean difference in exposures was small mean of 687 more sheep dipped (SD4504) handling of concentrate 9.9 more times (SD 44.5). The authors felt these changes could have arisen by chance. 8/43 reported change in pesticide between the two years (7 with type unknown in one of the two years). No change from non-OP to an Op-based dip. <u>Survival analysis</u> The time taken from first dipping to development of dippers flu was calculated for those starting to dip after 1969 (date of last dipping without dippers flu). In chronically unwell series there was no relationship with latency (RR 1.02 (95% CI 0.57-1.81) In the series without chronic illness the ratio was raised (RR 2.49 (95%CI 1.03-6.02) with those having an R allele at position 192 having a less favourable survival curve (figure reproduced in article). The six subjects with two R alleles had a slightly larger relative risk (2.74) than those with one R allele but this difference did not approach significance.		
Manthripragada	2010	STUDY OF PON1 POLYMORPHISM (PON1-55) AND OCCURENCE OF PARKINSON'S DISEASE EXPOSURE: The number of OPs to which residential exposure occurred was reported. Low/high residential exposure categories used. Occupational exposure determined as not exposed, possibly exposed, and likely exposed. The study group was predominantly white (80%)The average age at onset of PD was 68 years. Cases and controls had similar frequencies of occupational exposures (likely vs not exposed, adjusted OR = 1.08 (95%CI 0.74-1.56). Cases were more likely to be residentially exposed to organophosphates other than diazinon, chlorpyrifos and parathion for up to 3 vs more than 3 OPs (OR= 1.48 (95%CI 1.02-2.15)) Data pertaining to these results tabulated below.	<u>Strengths</u> Polymorphism (PON1 55) data for residents exposed to OPs, with objective measure of exposure. <u>Limitations</u> Authors note poor response of controls and possible selection bias. However this	OP exposure was associated with an increased risk of PD in subjects carrying PON1-55 MM genotype. This is consistent with evidence suggesting reduced metabolism of oxon OP metabolites by

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
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<td>
<table>
<tr><td></td><td>Cases (n=351) No (%)</td><td>Controls (n=361) no (%)</td></tr>
<tr><td>Sex (male)</td><td>199 (57)</td><td>179 (49)</td></tr>
<tr><td>Age</td><td></td><td></td></tr>
<tr><td>≤60 years</td><td>77 (22)</td><td>103 (28)</td></tr>
<tr><td>>60 years</td><td>274 (78)</td><td>260 (72)</td></tr>
<tr><td>Race</td><td></td><td></td></tr>
<tr><td>White</td><td>282 (80)</td><td>290 (80)</td></tr>
<tr><td>Black</td><td>3 (1)</td><td>14 (4)</td></tr>
<tr><td>Latino</td><td>46 (13)</td><td>32 (9)</td></tr>
<tr><td>Asian</td><td>4 (1)</td><td>11 (3)</td></tr>
<tr><td>Native American</td><td>16 (5)</td><td>16 (4)</td></tr>
<tr><td>County</td><td></td><td></td></tr>
<tr><td>Fresno</td><td>159 (45)</td><td>142 (39)</td></tr>
<tr><td>Kern</td><td>122 (35)</td><td>142 (39)</td></tr>
<tr><td>Tulare</td><td>70 (20)</td><td>79 (22)</td></tr>
<tr><td>Education</td><td></td><td></td></tr>
<tr><td>0-<12 years</td><td>64 (18)</td><td>37 (10)</td></tr>
<tr><td>12 years</td><td>97 (28)</td><td>73 (20)</td></tr>
<tr><td>>12 years</td><td>190 (54)</td><td>253 (70)</td></tr>
<tr><td>Cigarette smoking</td><td></td><td></td></tr>
<tr><td>Never</td><td>186 (53)</td><td>162 (45)</td></tr>
<tr><td>Current</td><td>21 (6)</td><td>33 (9)</td></tr>
<tr><td>Former</td><td>144 (41)</td><td>168 (46)</td></tr>
<tr><td>Occupational pesticide exposure *</td><td></td><td></td></tr>
<tr><td>Not exposed</td><td>213 (61)</td><td>239 (66)</td></tr>
<tr><td>Maybe exposed</td><td>22 (6)</td><td>24 (7)</td></tr>
<tr><td>Likely exposed</td><td>116 (33)</td><td>100 (28)</td></tr>
<tr><td>OP exposure*</td><td></td><td></td></tr>
<tr><td>0-< 3 OPs</td><td>238 (68)</td><td>275 (76)</td></tr>
<tr><td>≥3 OPs</td><td>113 (32)</td><td>88 (24)</td></tr>
</table>
*Classification based on occupational codes and self-reported agricultural and ground maintenance pesticide applications
* Greater than median exposure to 3 or more OPs, excluding diazinon, chlorpyrifos and parathion, between 1974-1999.

PON1-55 met hardy-Weinberg expectations in controls (P=0.52) The frequency of PON1-55 MM homozygous (but not heterozygous) variant genotype was higher among cases compared to
</td>
<td>was thought unlikely to affect PON1-55. Possible misclassification of exposure due incomplete address, geocoding difficulties, differences in wind patterns during/after pesticide applications, amount of time spent at home, introduction of pesticides into the home.</td>
<td>these subjects.

It is noted that there is a body of literature on PON polymorphisms and PD which wasn't captured by the literature search, probably because these papers refer to pesticide exposure in general rather than to OPs. Dick FD and colleagues (Occ Env Med, 64, 673-680, 2007) reported a gene-environment association for PD (solvents/GSTM1) but not significant associations for pesticides/PON1 and PD. (OR 4.43 (0.88-22.31 for PON L55M)</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>controls.

<table>
<tr><td></td><td>Cases No (%)</td><td>Control No (%)</td><td>Unadjusted OR</td><td>Adjusted OR*</td></tr>
<tr><td>PON 1 -55</td><td></td><td></td><td></td><td></td></tr>
<tr><td>LL</td><td>159 (45)</td><td>180 (50)</td><td>1.00</td><td>1.00</td></tr>
<tr><td>LM</td><td>144 (41)</td><td>148 (41)</td><td>1.10 (0.81-1.51)</td><td>1.04 (0.75-1.44)</td></tr>
<tr><td>MM</td><td>48 (14)</td><td>35 (10)</td><td>1.55 (0.96-2.52)</td><td>1.45 (0.87-2.40)</td></tr>
<tr><td>LL+LM</td><td>303 (86)</td><td>328 (90)</td><td>1.00</td><td>1.00</td></tr>
<tr><td>MM</td><td>48 (14)</td><td>35 (10)</td><td>1.49 (0.94-2.36)</td><td>1.43 (0.88-2.30)</td></tr>
</table>
* Adjusted for age, sex, ever smoked, race, county, education.

The authors reported an increased risk of PD associated with high level exposure to diazinon and chlorpyrifos.

The results of joint effects of PON1-55 genotype and OP exposure are given below.

The authors reported a 2-fold risk for diazinon exposed subjects with MM genotype compared with persons with wild type or heterozygous or no diazinon exposure (OR = 2.24 (95%CI 1.12-4.48))
This risk increase was evident among white people alone (2.68 (1.23-5.83)) even when adjusting for other OPs (2.43 (1.04-5.15). For those highly exposed to diazinon, an even larger increase in risk for MM carriers was reported (OR=5.30 (1.71-16.4)) and a strong interaction on the multiplicative scale (OR=4.59 (1.37-15.4)) The association was stronger when the assessment was restricted to white people only, based on small number of highly exposed (zero/low vs high OR=9.1 (2.0-41)) Effect estimates were similar for those ≤60 and >60 years.

Carrying MM genotype and exposed to chlorpyrifos was associated with an increase risk of PD compared with unexposed wildtype/heterozygous PON1-55 carriers (OR=2.61 (1.25-5.44), white only (2.95 (1.31-6.64)). The effect was slightly reduced when adjusted for other OPs (among whites 2.76 (1.16-6.15)) There was a slight increase in risk in LL and LM carriers (1.48 (1.04-2.12), whites only (1.48 (1.00-2.17) There was a stronger association in subjects ≤60 years (unadjusted OR=5.30(1.17-16.4)) (OR adjusted for other OPs=6.14 (1.34-28.05)); OR for >60 years adjusted OR=2.36 (0.97-5.79))

No associations between parathion and PD even for exposed PON1-55 MM genotypes carriers.

Results for pesticide main effect and interaction did not change appreciably when those with missing pesticide exposure were included as unexposed.</td><td></td><td></td></tr>
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<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5">7.B.3. Cross-sectional studies</td></tr>
<tr><td>Lee</td><td>2003</td><td>CHRONIC ILLHEALTH IN FARMWORKES AND ASSOCIATION WITH PON1 POLYMORPHISIMS

EXPOSURE: All 100 farm workers in this study were reported to have been exposed to OPs, but no details of exposure were given.

HEALTH OUTCOMES; Of the 100 subjects, 50 reported toxic symptoms as tabulated below

<table>
<tr><td>Variable</td><td>≤1 Toxic symptom</td><td>≥2 Toxic symptoms</td></tr>
<tr><td>Age (years)</td><td>32.2 (8.4)</td><td>32.8 (7.2)</td></tr>
<tr><td>Time as farm worker (years)</td><td>14.8 (9.9)</td><td>15.7 (7.6)</td></tr>
<tr><td>Smoking (pack-years)</td><td>8.7 (6.3)</td><td>11.0 (16.0)</td></tr>
<tr><td>Serum albumin (mg/dl)</td><td>46.8 (3.1)</td><td>46.0 (2.6)</td></tr>
<tr><td>Serum GGT (U/L)</td><td>20.5 (10.9)</td><td>20.2 (15.7)</td></tr>
<tr><td>Cholinesterase (plasma U/L)</td><td>6745 (1407)</td><td>6308 (1382)</td></tr>
<tr><td>Cholinesterase (RBC U/L)</td><td>36.3 (4.1)</td><td>36.8 (4.4)</td></tr>
<tr><td>7 or more years at school %</td><td>42.0</td><td>44.0</td></tr>
<tr><td>Current alcohol use %</td><td>76.0</td><td>84.0</td></tr>
<tr><td>Positive for two or more alcohol abuse questions %</td><td>56.0</td><td>74.0*</td></tr>
<tr><td>History of head injury %</td><td>32.0</td><td>56.0**</td></tr>
<tr><td>Neurologic symptom score</td><td>0.4 (0.5)</td><td>4.1 (2.3)***</td></tr>
<tr><td>History of acute pesticide poisoning (%)</td><td>2.0</td><td>8.0</td></tr>
</table>
* P=0.06, ** P=0.02, *** P=0.0001

As expected the neurologic symptom score was higher in those reporting symptoms

Of the subjects in the study, 49 were pesticide applicators. As expected the proportion of subjects with symptoms was significantly higher among applicators than non-applicators of pesticides (63.3% v 37.3%, OR=2.9 (95%CI 1.3-6.5, p=0.009)</td><td>Strengths Subjects not aware of genotype at time toxic symptoms were recorded.

Limitations. Selected small group of exposed/non-exposed subjects (100)from an initial cohort of 347 subjects. Authors argue that non-exposed may have had some exposure to pesticides, hence occurrence of toxic symptoms in non-applicators.
No specific measure of paraoxonase activity in these farm workers was reported.</td><td>The authors conclude that the prevalence of chronic toxicity symptoms increased in a stepwise manner according to ‘slow ‘PON192 genotypes. (specifically the occurrence of the PON192 Q allele)</td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		Of the 100 subjects 82 underwent genotyping. The frequency of Gln/Gln (Q/Q), Gln/Arg (Q/R), and Arg/Arg (R/R) were 11, 45, and 43% respectively (reported to be different from Caucasian population) Subjects with Arg/Arg (R/R) were classified as fast genotype (37). Those with Gln/Arg (Q/R) and Gln/Gln (Q/Q) were combined as slow genotype (n=45, 60%) A significantly higher proportion of subjects with the slow genotypes reported two or more symptoms of chronic toxicity compared to those with fast PON activity. (OR 2.8 (95%CI 1.1-6.7)) There was no association between PON and dummy symptoms (chest pain, ear ache). The samples from remaining 18 subjects did not contain sufficient DNA for genotyping. Subjects did not differ significantly in characteristics compared to the subjects where genotyping had been undertaken. A figure was reported regarding the interaction among genotype, pesticide exposure status, and chronic pesticide toxicity. The proportion of subjects with toxic symptoms increased in a step-wise fashion from non-applicator-fast genotype (15%), non-applicator-slow-genotype (42.9%), -applicator -fast genotype (58.8%), applicator-slow genotype (75%)..(Mantel-Haenszel Trend test p=0.001) In multivariable logistic regression analysis, the independent predictors of chronic pesticide toxicity were previous history of head injury (2.8 (95%CI 1.7-4.7), slow genotypes (2.9 (1.7-4.9) and history of having worked as a pesticide applicator (OR 5.1 (95%CI 3.2-8.9))		
Browne	2006	RELATIONSHIP OF EEG AND LORETA TOMOGRAPHY TO PON1 STATUS IN OP EXPOSED RURAL COMMUNITY EXPOSURE: Exposure to cholinesterase inhibitors confirmed at all distances (20m intervals from ticket placed in field being sprayed) from the field where spraying occurred.(AGRI Screen tickets which respond colourimetrically from low parts per billion to 6 ppm) OPs routinely used included fenitrothion, chlorpyrifos, monocrotophos, ethion and azinphos-methyl) HEALTH OUTCOMES: With regard to the 30 subjects with biochemical data. There was a significant reduction in AChE activity in exposed (41% lower than control, p<0.001) No significant difference in BuChE.	Strengths. Measured both paraoxonase activity and investigated PON1 genotypes in exposed and non exposed subjects. <u>Weaknesses</u> The rationale for a 4 fold increase in PON1 activity in exposed is	P192 R genotype was found to be associated with increased Paraoxonase activity and abnormal EEG patterns. The authors did not provide a coherent rationale as to why increased

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		PON1 and arylesterase were significantly higher in exposed 447% and 441% of control p<0.001. Activities of these two enzymes were not significantly different between exposed agricultural workers (9) and exposed local rural population (20) Allele frequency in study population was similar to that of the Israeli population for PON1 55, PON1 192 and AChE 17130ΔHNF3β. There was a higher frequency in this study regarding PON1 108 C allele than in the general Israeli population (0.70 compared to 0.40). In general AChE activity did not vary by PON1 genotype except for a slight decrease in LLRR compared with MMQQ (180.97±8.30041, compared to 216.6±9.16877, P=0.04) PON1 genotypes correlated with significant alterations in paraoxonase activity. The presence of PON1 L to M substitution (TTG to ATG) was consistent with reduced PON1 activity. The authors controlled for effects of PON1 Qi192 R substitution by selecting individuals without the R allele. In non-exposed MMQQ (10 subjects) exhibited one-third of the activity of LLQQ subjects (14). (P<0.0001) In contrast in exposed subjects MMQQ (6) and LLQQ(7) were almost equal activity (ratio 1.3) The authors found that MM exposed individual had 8.1x PON1 activity than controls. The authors found that LL exposed individuals had 3.6x PON1 activity than controls. With regard to PON1 Q192R (CAA to CGA) substitution . In controls there was a gene-dependent increase in PON1 correlated with R allele (PON activity nmol/min*ml) QQ 29.6, QR 71.3, RR 99.9. slope35.18, R^2 0.9886 The authors reported more robust changes in exposed subjects QQ 150.7, QR 384.0, RR 513.7, slope 181.48, R^2 0.9735 the authors controlled for effect of PON L55M substitution by selecting individuals without the M allele and reported R allele associated changes in PON activity remained. The authors identified some further differences between exposed and controls. Non-exposed LLRR individuals (n=13) had approximately 2x more PON1 activity than LLQQ individuals (n=10) (ratio 2.09, P<0.0001) In exposed LLRR subject (n=3) showed 3 times more PON activity than LLQQ individuals (n=7) (ratio 2.86, P<0.0001). the authors suggest an exposure-dependent response associated with PON1 Q192R substitution and a potential biosensor effect of the R allele in anti-AChE exposure. The authors hypothesised that increased beta3 EEG activity may differ among exposed individuals with different genotype profiles. Significantly increased beta3 activity in frontal cortical regions and decreased activity in the	not clear. Genotype affected PON1 activity in both exposed and controls. The authors report association of PON1 192 R allele with increased beta3 EEG activity and concurrent changes in tomography in specific brain regions. It is unclear how increased paraoxonase activity could be associated with the changed reported.	paraoxonase activity could be related to deleterious effects.

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		temporal regions in exposed individuals with the R allele compared to controls and exposed without the R allele. Comparing LORETA values (representing source densities) in these brain regions revealed exposed with R allele exhibited significant frontal activity and decreased temporal activity (P<0.03). Non-exposed and exposed without R allele had similar LORETA patterns.		

Table 8. EXPOSURE SCENARIO AND POLYMORPHISM / ADVERSE EFFECTS

B. CHRONIC LOW-LEVEL EXPOSURE

Table 8.B.2. Case-Control Studies

Test Name / Diagnosis	First Author and Date					
	Cherry 2002 Sheep Dippers	Mackness 2003 Sheep Dippers	Povey 2005 Sheep Dippers (Exclusion of subjects to form homogeneous groups)	Povey 2007 Sheep Dippers	Cherry 2011 Sheep Dippers	Manthripragada 2010 Rural community US
PON1 -55 LL associated with self-reported chronic toxicity	+	+	+			
PON1 -55 MM associated with Parkinsons' disease						+
PON1-192 RR associated with self-reported chronic toxicity	+	+	+			
PON1-192 RR associated with 'Dippers' flu'					+	

Test Name / Diagnosis	First Author and Date					
	Cherry 2002 Sheep Dippers	Mackness 2003 Sheep Dippers	Povey 2005 Sheep Dippers (Exclusion of subjects to form homogeneous groups)	Povey 2007 Sheep Dippers	Cherry 2011 Sheep Dippers	Manthripragada 2010 Rural community US
PON1-55 LL associated with 'Dippers' flu					-	
Low diazoxonase activity in PON1-192 RR subjects	+	+			+	
High paraoxonase activity in PON1-192 RR subjects		+				
GSTM1, GSTP1, GSTT1 associsted with self-reported chronic toxicity				-		

Table 8.B.3. Cross-Sectional Studies

Test Name / Diagnosis	First Author and Date	
	Lee 2003 South African farm workers	Browne 2006 Rural community Israel
PON 1-192 QQ or QR reported with two or more signs of OP chronic toxicity.	+	
PON1-192 R Significant beta3 activity in frontal cortical regioins. Decreased activity in temporal regions.		+
PON1 192 R Significant changes in brain tomography		+
Higher paraoxonase in exposed subjects		+

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF 'OTHER' NEUROLOGICAL EFFECTS

1. This section considers neurological symptoms observed and analysed in 13 studies of the review which do not fit into the specific sections of Peripheral Neuropathy or Autonomic Symptoms, but which require attention for the information they may provide of other neurological disorders. The most common symptoms included are headache, fatigue or tiredness, lack of energy, and eye symptoms such as tiredness of the eyes, blurred or dim vision or eye irritation. Further symptoms described in just one or two studies include difficulty concentrating, trouble remembering things, difficulty writing or speaking, increased sensitivity to chemicals or changes in smell or taste, and clustering together of neurological symptoms. It will be considered whether the report of this range of symptoms provides useful information in the overall evaluation of the effects of OPs.

ACUTE EXPOSURE WITH CHRONIC EFFECTS

2. Six studies reported symptoms in this category: 2 cohort studies, Kawana 2001 and Nakajima 1999, and 4 cross-sectional studies, McCauley 2001 and 2002, Ohtani 2004 and Stallones 2002b (Annex 11, Table 6).

Symptoms of Fatigue, Tiredness or Weakness

3. Four of the studies reported on general fatigue, tiredness or weakness. McCauley 2001 reported persistent fatigue which was found to be significant both in troops deployed to the Gulf during the Gulf War and troops in the vicinity of, or involved in, the detonation of chemicals at a munitions dump in Khamisiyah, Iraq, containing sarin and cyclosarin (Annex 11, Table 7.A.3, McCauley 2001, "Neurological symptoms reported by Gulf War veterans 9 years after destruction of munitions dump at Khamisiyah"). Ohtani 2004, reporting on the effects of the Tokyo subway release of sarin 5 years after the event, reported that over 40% of the group of victims studied were easily fatigued, but the study provided no further information or analysis than a percentage of affected individuals (Annex 11, Table 7.A.3, Ohtani 2004). Nakajima 1999, also reporting on the effects of sarin exposure, though in a residential district in Matsumoto, reported a significant prevalence of fatigue at 1 year after the incident, but a non-significant result at 3 years afterwards (Annex 11, Table 7.A.1, Nakajima 1999,"Prevalence of symptoms 1 year after sarin exposure in Matsumoto incident" and "Neurological symptoms 3 years after sarin exposure in Matsumoto incident"). Stallones 2002b reported on acute exposure to OP pesticides and the longer-term effects of pesticide-related illness, and did not find the symptom "tired more easily than usual" to be significant (Annex 11, Table 7.A.3, Stallones 2002b, "Neurological symptoms associated with having had a pesticide-related illness"). The four

studies each reported very different circumstances of exposure and, overall, provided insufficient and inconclusive evidence to suggest that fatigue might be a long-term effect of an acute exposure to OPs.

Symptom of headache

4. Two of the studies from paragraph 2 also reported on headache as a chronic effect of acute OP exposure. Ohtani 2004 stated that 44.8% of the group of victims of the Tokyo subway attack that they studied reported suffering from headache at 5 years after the incident (Annex 11, Table 7.A.3, Ohtani 2004). Nakajima 1999, on the other hand, reported non-significant findings for headache in victims of the Matsumoto incident at 1 and 3 years after the event (Annex 11, Table 7.A.1, Nakajima 1999,"Prevalence of symptoms 1 year after sarin exposure in Matsumoto incident" and "Neurological symptoms 3 years after sarin exposure in Matsumoto incident"). These results were inconclusive, but headache was also reported as an effect of chronic low-level exposure by 3 other studies (paragraph 17).

Eye symptoms

5. Two studies that followed up victims of the Tokyo subway sarin attack who were treated at St Luke's Hospital in Tokyo reported responses about eye symptoms from the questionnaires used in the studies. Kawana 2001 reported on a large group of 582 sarin patients, while Ohtani 2004 reported only on 34 victims, and it was not clear if these were part of the larger group in the Kawana study. The large Kawana study reported that results for tiredness of eyes, dim vision, and difficulty focusing were not significant at the $p<0.05$ level (Annex 11, Table 7.A.1, Kawana 2001, "Frequency and percentage of symptoms in St. Luke's Hospital sarin patients"), whereas Ohtani stated that 63.3% of their subjects reported blurred vision, 60% difficulty in focusing, and 56.7% eyes becoming easily tired (Annex 11, Table 7.A.3, Ohtani 2004). The Kawana study would appear to be more reliable, based on large study size and the fact that the results were tested for significance with *p* values.

Miscellaneous symptoms

6. A few symptoms were reported in only one study. McCauley 2001 reported significant results for increased sensitivity to everyday chemicals, and unexplained weight gain, in Gulf War veterans potentially exposed to sarin through the Khamisiyah incident in Iraq ((Annex 11, Table 7.A.3, McCauley 2001, "Neurological symptoms reported by Gulf War veterans 9 years after destruction of munitions dump at Khamisiyah"). Stallones 2002b reported difficulty concentrating, having to make notes to remember things, and difficulty understanding the meaning of newspapers and books as significant results for subjects who experienced an acute pesticide-related illness (Annex 11, Table 7.A.3, Stallones 2002b, "Neurological symptoms associated with having had a pesticide-related illness"). A highly significant result was obtained for relatives noticing that the subject has trouble remembering things, with a χ^2 test for trend of 22.40, P value <0.0001. These were isolated reports of each symptom, and, on their own, were insufficient evidence that these symptoms may be chronic effects of acute exposure to

OPs. Furthermore, the Stallones study found the association with pestcides, not specifically OPs, and the connection with OPs is indirect, through the finding that OPs applied to crops were associated with a pesticide-related illness, so the highly significant symptom may be related to pesticide exposure more than specific OP exposure.

Conclusions on Acute Exposure with Chronic Effects

7. The six studies in this section reported diverse effects potentially related to acute exposure to sarin, and, in only one study (Stallones 2002b), to pesticides including OPs. It was difficult to draw any firm conclusions regarding significance of the findings on the limited data available for each symptom. The symptom of headache was reported by further studies in the chronic low-level exposure section (paragraph 17).

EFFECTS OF CHRONIC LOW-LEVEL EXPOSURE

8. Seven studies reported symptoms in this category. They were: 1 cohort study, Albers 2004b, and 6 cross-sectional studies, Bazylewicz-Walczak 1999, Cox 2005, Kamel 2005, Kilburn 1999, Lee 2003, and Solomon 2007 (Annex 11, Table 11.B).

Chronic Organophosphate Induced Neuropsychiatric Disorder (COPIND)

9. In the 1999 COT report, a description was given of a postulated Chronic Organo-Phosphate Induced Neuropsychiatric Disorder, based on surveys reported in Davies 1999. Davies listed 10 symptoms considered to make up COPIND: exacerbation of dipper's flu (ill health experienced by some farmers within days of dipping sheep); personality changes; impulsive suicidal thoughts; cognitive impairment; language disorder; alcohol intolerance; heightened sense of smell; handwriting deterioration; sensitivity to OPs; decreased exercise tolerance. According to the publication author, a patient showing at least seven of these symptoms would be diagnosed as having COPIND. The author explained COPIND as a characteristic pattern of neurological symptoms in patients with psychiatric problems who had had long-term OP exposure (COT 1999 Report, paragraph 7.62 (Annex 16)).

10. COPIND is not a generally recognised disorder, and has been postulated mainly by Davies and co-workers Ahmed and Freer. The one study which set out to examine the possible existence of such a disorder, as well as to analyse neuropsychiatric symptoms in users of sheep dip and other pesticides, was Solomon 2007. This was a large population-based survey of 9,844 men resident in three rural areas of England and Wales, divided into 3 exposure groups: those who had worked with sheep dip (OPs); those who had worked with other insecticides but never with sheep dip; and those who had worked with other pesticides but never sheep dip or insecticides. A large reference population of 6,109 men resident in three rural areas of England and Wales was included (Annex 11, Table 6.B.3, Solomon 2007). The aim of the study was to look for evidence of increased prevalence or unusual

clustering of postulated COPIND symptoms in subjects who had worked with sheep dip, compared to subjects in the other categories of pesticide exposure.

11. The findings of this large and detailed cross-sectional study concerning neurological symptoms are described in Annex 11, Table 7.B.3, Solomon 2007. The occurrence of two psychiatric symptoms, anxiety and depression, in the study population was described in the Psychiatric Illness annex, specifically in Annex 6, Table 7.B.3, Solomon 2007, and associated summary paragraph 38.

12. Solomon 2007 considered the prevalence of 7 neurological symptoms, which were not exactly the same as those listed by Davies. They were: difficulty concentrating; difficulty remembering things; difficulty with handwriting; difficulty speaking; sensitivity to certain smells; increased sensitivity to effects of alcohol; and tiredness and lack of energy. Men who had worked with sheep dip had a higher prevalence of all 7 symptoms, compared to men who had never worked with pesticides, but the symptoms were also found to be more frequent in men who had worked with other pesticides, not sheep dip (Annex 11, Table 7.B.3, Solomon 2007) Prevalence of neurological symptoms according to previous work with pesticides"). There was also strong evidence of clustering of symptoms (Annex 11, Table 7.B.3, Solomon 2007) Clustering of neurological symptoms according to previous work with pesticides"). However, the clustering was not specific to sheep dip or to insecticides, and there was no indication of a different pattern of clustering among men who had worked with sheep dip compared to those who had worked only with other pesticides, or not with pesticides at all.

13. Several further analyses were carried out in the Solomon 2007 study. The authors derived a "somatising tendency" score from the Brief Symptom Inventory, a test used to assess patients for psychological problems (description in Annex 6, Table 9). "Somatisation" is a tendency to experience medically unexplained symptoms, and one analysis was performed to look for possible association between somatising tendency and symptoms (Annex 11, Table 7.B.3, Solomon 2007, 3) "Associations of neurological symptoms with occupational exposure to pesticides and somatising tendency"). Reporting multiple neurological symptoms, in particular more than 4, was strongly associated with somatising tendency, but no consistent association was observed with the different classes of pesticides. However, the strongest association for reporting 4 or more symptoms was with sheep dip, with a Prevalence Ratio of 1.4, 95% CI 1.0-2.0.

14. A further analysis looked specifically at users of sheep dip. It was found that experiencing multiple neurological symptoms was more common among men who had experienced fever or chills or more than 4 neurological symptoms within 48 hours of using sheep dip (the postulated "dipper's flu" response to sheep dipping) (Annex 11, Table 7.B.3, Solomon 2007) "Associations with neurological symptoms among past users of sheep dip"). However, handling sheep dip concentrate, which suggests higher exposure to the OPs among users, was not found to be significantly associated with developing neurological symptoms. Another analysis found that symptoms of difficulty speaking and difficulty with handwriting were marginally higher

among men who had worked with sheep dip than in those who had never worked with pesticides (Annex 11, Table 7.B.3, Solomon 2007) “Lifetime prevalence for consulting a GP for 2 neurological symptoms, according to previous work with pesticides”).

15. Overall, however, the associations noted in Solomon 2007 were not specific to sheep dip: clustering of the 7 neurological symptoms was more common among past users of sheep dip, but not specific to such users. Similarly, reporting multiple neurological symptoms was more common in past users of OPs, but the association was not specific to users of sheep dip. Handling sheep dip concentrate was not significantly associated with developing neurological symptoms. Such evidence does not appear to support the existence of COPIND, as symptoms are not specifically OP-induced.

Symptoms of Fatigue and Lack of Energy

16. Only one study, Cox 2005, which evaluated potential health effects on residents from spraying of methyl parathion in the home, reported significant results for fatigue and lack of energy as long-term sequelae of the exposure (Annex 11, Table 7.B.3, Cox 2005). Memory problems were not reported to be significant.

Symptom of Headache

17. Recurring headache resulting from chronic exposure was reported by 3 studies, Cox 2005, Kamel 2005, and Kilburn 1999. The Cox and Kilburn studies both related to indoor spraying of an OP pesticide (methyl parathion in the Cox study, chlorpyrifos in Kilburn), while Kamel related to outdoor agricultural use of OPs. Kamel was a large study, using data from the Agricultural Health Study cohort, and included information from 18,782 pesticide applicators. Two studies reporting on chronic effects of acute exposure (paragraph 4) also found significant results for headache, so there is a suggestion that recurring or persistent headache may be associated with OP exposure, although more results might be needed to properly verify this finding.

Miscellaneous Symptoms

18. Changes in smell or taste were reported by Kamel 2005, Kilburn 1999 and Solomon 2007. Difficulty speaking was reported by Kamel as well as Solomon. Kamel also reported the eye symptoms of blurred vision and poor night vision. Kilburn was the only study to report a group of respiratory and chest symptoms, these being chest tightness, palpitations, burning in the chest, and shortness of breath (Annex 11, Table 7.B.3, Kilburn 1999). There were inconsistent results for grouped neurological symptoms: Solomon 2007 found evidence for clustering of neurological symptoms, as did Lee 2003, but the much smaller Bazylewicz-Walczak 1999 study did not find a significant combined neurological symptom score.

Conclusions on Effects of Chronic Exposure

19. The major study in this section was Solomon 2007, which examined aspects of the postulated COPIND disorder and presented evidence that the neurological symptoms and their clustering were not specific to sheep dip or to insecticides, and therefore casting some doubt on the grouping of these symptoms and naming them as a specific disorder. There was little evidence relating to other neurological symptoms and their being consistently associated with low level chronic OP exposure.

Table 6. 'OTHER' NEUROLOGICAL STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
A. Acute Exposure with Chronic Effects									
6.A.1. Cohort studies									
Kawana	2001	Japan	582 sarin patients given emergency treatment at St Luke's International Hospital, Tokyo, on day of the sarin subway attack (20 March, 1995)	655 victims of Tokyo sarin attack studied by a non-governmental organisation; 88 victims of the 1994 Matsumoto sarin attack; 87 Matsumoto controls	Symptoms reported on St. Luke's Questionnaire, developed by the investigators; contained 33 five-choice Likert scale items; questions included physical and eye symptoms which could be indicative of neurological dysfunction	Symptoms of dizziness, tiredness of eyes, dim vision and difficulty focusing	Release of sarin in the subway; the quantity that individuals were exposed to is not known	Not considered	symptom frequencies and percentages for 1997, 1998 and 2000 reported for sarin patients, comparisons made using Fisher's exact test; *p* values of <0.05 indicated; symptom frequencies also compared between 1997 and 1998, and 1998 and 2000, using Fisher's exact test, and *p* values of <0.1 indicated; comparison of symptom incidence between St Luke's patients and the three reference populations (percentages reported)
Nakajima	1999	Japan	First survey conducted 3 weeks after the attack, and involved 2052 residents in an area 1050 metres north to south and 850 metres east to west where about 12 litres of sarin were released on the night of June 27, 1994,in	919 out of the 1237 respondents to the 1 year survey who were classified as non-victims of the sarin attack, because they did not have a diagnosis of sarin toxicity and did not experience muscarinic and/or nicotinic symptoms immediately after the sarin incident;	Questionnaires at 1 year and 3 years after the attack. Questions asked respondents whether they were given a diagnosis of sarin toxicity or had experienced muscarinic and/or nicotinic symptoms, and also asked about hospitalisations and consultations with doctors after the sarin attack;	Fatigue and headache	It is known that about 12 litres of sarin were released in the specific area of Matsumoto City, but individual exposure, or proximity of individuals to the release, were not measured; 93 persons had cholinesterase activity measured 4 weeks after the	Not considered	The significance of prevalence of symptoms subjectively described 1 year after the sarin exposure was tested by the χ^2 test; the relationship between cholinesterase activity 4 weeks after sarin exposure and symptoms 1 year after the event was tested by One-way ANOVA, *p* values reported; differences in prevalence of symptoms

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
			Matsumoto City, with the sarin release site in the centre; results of first survey reported in a previous paper; Survey conducted 1 year after the incident, with 1237 respondents out of the original 2052; 318 of the 1237 were sarin victims, defined as such either because they were given a diagnosis of sarin toxicity, or experienced mucsarinic and/or nicotinic symptoms immediately after the attack; for the 3 year survey, 2000 persons living in the same area about 3 years after the incident were approached; there were 836 respondents, of whom 167 were classed as victims	for the 3 year survey, 669 people out of the 836 respondents were classed as non-victims	the 1-year survey contained 13 questions regarding specific symptoms, and whether those had been experienced within the preceding month; the same 13 symptoms were asked about in the 3-year survey		sarin exposure and again for the 1 year survey		between victim and non-victim groups in the 3 year survey evaluated using χ^2 test or Fisher's exact test if number in any one group too small; ORs and 95% CIs for association between sarin exposure and symptoms 3 years after the sarin incident reported

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
6.A.2. Case-control studies - none									
6.A.3. Cross-sectional studies									
McCauley	2001	Study done in the USA, exposure thought to have occurred at Khamisiyah, Iraq, where a munitions dump was destroyed	653 Khamisiyah veterans who were operating within a 50 km radius of the Khamisiyah Ammunition Storage Point where the munitions were destroyed; veterans had to be resident in one of the 5 states of Oregon, Washington, California, North Carolina or Georgia	610 Non-Khamisiyah military personnel who were deployed in the Gulf during the Gulf War, but were outside the 50 km radius and had no known exposure to nerve agents; 516 Non-Deployed personnel who had been on active duty at the time of the Gulf War, but not deployed to the Gulf; participants were also residents of one of the 5 states of Oregon, Washington, California, North Carolina or Georgia	Self-reported symptoms on a questionnaire; Computer Assisted Telephone Interviews conducted	Persistent fatigue, tiredness or weakness; increased sensitivity to everyday chemicals, coninuous eye irritation or sensitivity; unexplained weight loss or weight gain	No direct measure; exposure was assumed for troops within a 50 km radius of the detonated chemical warfare agents. Self-reported information on personnel movement in the Khamisiyah area included	ORs and 95% CIs adjusted for potential confounders of age, gender, and region of residence; selection bias possible because sample limited to persons whose telephone numbers could be tracked down by common search mechanisms; the Khamisiyah population of veterans was not evenly distributed throughout the US, so there may be bias due to location; also possible misclassification, because 50 subjects considered by the Department of Defense to be in the Khamisiyah-deployed group reported they had never been in Iraq	Logistic regression, reporting ORs and 95% CIs; categorical data analysed using χ^2 tests, and the continuous variable of age analysed using *t*-tests

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
McCauley	2002	As above	As above	As above	Self-reported conditions diagnosed by a physician since the Gulf War; Computer Assisted Telephone Interviews conducted	Epilepsy or seizures, and any other neurological disease not reported under Peripheral Neuropathy or Autonomic Symptoms	As above	As above	Frequency of diagnosed medical conditions in Gulf War veterans reported; ORs and 95% Cis calculated for the likelihood of reporting medical conditions diagnosed by a physician since the Gulf War
Ohtani	2004	Japan	34 victims (20 males, 14 females, 21-69y, mean age male 50.2±11.9 y, female 39.6 ±10.4) of the Tokyo subway sarin attack treated at emergency wards for acute sarin intoxication at St. Luke's International Hospital in Tokyo; study conducted at Tokyo University	None for the 34 victims studied	Self-rating questionnaire evaluating 34 subjective somatic and mental symptoms, developed by staff at St. Luke's International Hospital	Blurred vision; difficulty in focusing; eyes become easily tired; difficulty in seeing far; headache; easily fatigued	Release of sarin in the subway; the quantity that individuals were exposed to is not known	Not considered	Frequency of physical symptoms, percentages reported
Stallones	2002b	USA	761 repsondents to the questionnaire concerning neurological symptoms and exposure to pesticides, including several specific OPs; 69 respondents reported a pesticide-related illness	692 of the respondents who did not report a pesticide-related illness	Self-reported pesticide poisoning and neurological symptoms on questionnaire	Getting tired more easily than expected; having difficulty concentrating; being confused or disoriented; having trouble remembering things; having to make notes to remember things; finding it hard to understand the meaning of newspapers, magazines and	Self-reported use of pesticides, including specific OPs	Regression analyses adjusted for age and gender; other co-variates considered were years of schooling, alcohol consumption and farm income; analyses relate to pesticide-related illness, not specifically OP-related	Univariate and multivariate conditional regression; stepwise logistic regression; ORs and 95% CIs reported; Mantel-Haenszel χ^2 test for trend for neurological symptoms significantly associated with acute pesticide-related illness, *P* values reported

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
						books that were read; relatives noticing that subject had trouble remembering things		illness, so co-exposure in respondents to pesticides other than OPs is likely	
6.A.4. Case series studies - none									
6.A.5. Case reports - none									
B. Chronic Low Level Exposure									
6.B.1 Cohort studies									
Albers	2004b	USA	All Dow employees engaged in the manufacture of chlorpyrifos (aged 18-65y) who were employed on 1 Sept 1999. Of 66 potentially eligible, 53 took part (80%). All these subjects took part in 1 year follow up	Dow employees engaged in manufacture of Saran (plastic film) with no known exposure to neurotoxicants. Out of 74 workers asked, 60 (81%) participated, with 58 also participating at 1 year follow-up	Neurological questionnaire with self-reported symptoms, clinical evaluation by board-certified neurologist	Aspects of Central Nervous System functioning not covered in other sections	Urinary excretion of OP metabolites measured; plasma butyrylcholinesterase and acetylcholinesterase measured	Chlorpyrifos exposed and referents were matched for age, height, weight, BMI, reading scale, smoking pack year and anxiety	Fisher's exact two-tailed test performed and *P*-values reported
6.B.2. Case-control studies - none									
6.B.3. Cross-sectional studies									
Bazylewicz-Walczak	1999	Poland	26 women working as greenhouse workers, performing standard gardening jobs, employed at 3 large gardening enterprises	25 women considered unexposed as the control group; these women were employed in canteens, kitchens and administrative jobs in the same 3 gardening	Symptom questionnaire that forms part of the Neurobehavioural Core Test Battery (NCTB) recommended by the WHO (Johnson ed., 1987), in a Polish adaptation – the Subjective Symptoms	From the Subjective Symptoms Questionnaire, questions relating to vertigo, trembling of the hands, dropping objects easily from the hands, finding it difficult to walk in the dark, experiencing	Air sampling carried out at 5 measuring sites in the greenhouses; concentrations of pesticides on clothing determined from pads placed on different parts of	Participants were matched with respect to sex, age, level of education and place of habitation; co-exposure of greenhouse workers to other	Two-way ANOVA performed to assess differences between exposed and control groups, *p* values reported

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
				enterprises	Questionnaire	changes in sense of smell or taste, and experiencing numbness or strange sensations in the muscles of the face	garments, and level of pesticides on the skin determined in washes from the skin	pesticides – carbamates, synthetic pyrethroids and dithiocarbamates	
Cox	2005	USA	121 subjects from 49 homes in the state of Mississippi with high levels of methyl parathion (MP), >150µg/100cm²	Control group of 170 subjects from 56 homes in state of Mississippi with non-detectable or very low levels of MP, <15µg/100cm²	Medical evaluation by physician blinded to level of MP in the home; medical history and physical examination included; children of 12 and under had growth evaluations performed	Most frequent neurological symptoms reported were headache, lack of energy, blurred vision, difficulty concentrating; also reported were weakness and memory problems	10cm-square wipe samples taken of flat surfaces in different rooms in each house, and results averaged; samples analysed for MP using gas chromatography and mass spectrometry	Evaluating physician judged whether signs or symptoms could be explained by other disease states, tobacco use, second hand tobacco exposure, or other situations	ANOVA and *t* tests to compare continuous data; Bonferonni test to determine significance in multiple groups; logistic regression to compare findings for groups, *p* values reported for frequency of symptoms between groups, and OR of increased frequency of symptoms in homes with highest levels of MP
Kamel	2005	USA	18,782 white male licensed private pesticide applicators, grouped into "Cases" and "Controls"; "Cases" were the 20% of applicators who experienced ≥ 10 symptoms in the year before enrolment, in summary measures of neurologic symptom prevalence	"Controls" were the 80% of applicators who experienced < 10 symptoms in the year before enrollment, in summary measures of neurologic symptom prevalence with pesticide use and exposure	Self-reported disease states reported in questionnaire	Headache, loss of appetite, poor night vision, blurred/double vision, difficulty speaking	Self-reported exposure	Adjustments made for age, state, education, smoking and alcohol use	Linear regression for number of symptoms and logistic regression for dichotomous outcomes; ORs for having ≥ 10 symptoms in the year before enrollment, and ORs for experiencing a specific symptom with high frequency compared with low frequency and OP exposure
Kilburn	1999	USA	22 chlorpyrifos-exposed subjects, aged 34-77 years,	264 men and women aged 18-83 years, unexposed to	Questionnaire developed by the study authors	Neurological symptoms that do not fall into other categories of this	Schedules of pesticide application were found for offices of	Age, education, gender, height, family income, hours of general	Symptom frequencies reported for exposed and unexposed, as means with standard

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
			seen in a neurotoxicology clinic between 1991 and 1997; they represent 6% of 384 patients evaluated for possible neurobehavioural effects of chemicals	neurotoxic chemicals, recruited at random from voter registration rolls in Wickenburg, AZ and Smithfield, LA.		review: headache, dizziness, light-headedness, decreased smell	6 patients, so concentrations of applied chlorpyrifos were known; 4 patients had personal clothing or rugs analysed for chlorpyrifos; for other subjects, self-reported exposure on questionnaire	anaesthesia, weight; pre-existing nervous system disease, head trauma	deviations; both compared by analysis of variance, and *p* values reported
Lee	2003	South Africa	100 farm workers selected from 347 subjects from 73 participating farms in Western Cape, South Africa; subjects genotyped for polymorphism of the PON gene at position 192; genotype information was available for only 82 of these. 49 of the subjects in the study were pesticide applicators	From the 73 participating farms, one non-applicator from the same farm was selected for every two applicators of pesticides, matched for age within 2 years and education within 1 year, in the original survey of 347 subjects	Questionnaire ; blood samples obtained for genotyping in the 100 selected subjects; blood samples also analysed for γ-glutamyl transferase, serum albumin (as marker of chronic undernutrition), and plasma and erythrocyte cholinesterase (as markers of acute OP exposure and toxicity, respectively)	Symptoms used to construct a neurologic symptom score were chronic abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbance, limb numbness, paresthesias, limb pain, limb weakness	Self-reported pesticide applicator history	Risk factors considered were age, number of years on job, smoking and alcohol history, education level, plasma or red blood cell cholinesterase level and previous history of acute OP poisoning; selection bias possible, because the 100 subjects chosen for genotyping were said to have been selected by the authors "by convenience"	2-tailed t-test for unpaired data, Wilcoxon rank sum test and Fisher exact test to compare means, Mantel-Haenszel test for trend; multi-variable logistic regression analysis perfomed, ORs and 95% CIs reported
Solomon	2007	UK	9844 men born between 1933-1977 resident in 3 rural areas of England/ Wales, of whom 1913 had at some time worked with	6109 men born between 1933-1977, resident in 3 rural areas of England/ Wales, who had never worked with any type of pesticide	Self-reported symptoms on questionnaire	Neurological symptoms associated with COPIND (chronic organophosphate-induced neuropsychiatric disorder)	Self-reported lifetime history of work with following five categories of pesticides (postal survey): sheep dip, other insecticides, herbicides,	Age, area, acute effects of exposure to pesticides in the year before completing questionnaire	Modified Cox regression; prevalence ratio (PR) with 95% CI

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors / bias / confounders	Statistical analysis
			sheep dip, 832 had worked with other insecticides but never with sheep dip,and 990 had worked with other pesticides but never with sheep dip or insecticides				fungicides and wood preservatives		
6.B.4. Case series studies - none									
6.B.5. Case reports - none									

Table 7. 'OTHER' NEUROLOGICAL RESULTS AND CONCLUSIONS

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td colspan="5">A. Acute Exposure with Chronic Effects</td></tr>
<tr><td colspan="5">7.A.1. Cohort studies</td></tr>
<tr><td>Kawana</td><td>2001</td><td>STUDY FOLLOWING UP VICTIMS OF THE TOKYO SUBWAY SARIN ATTACK TREATED AT ST LUKE'S HOSPITAL IN TOKYO, UP TO 5 YEARS AFTER THE INCIDENT

EXPOSURE Unknown quantity of sarin released in the Tokyo subway; symptoms self-reported on questionnaire

HEALTH EFFECTS The table below indicates the frequency of several eye symptoms included in the St. Luke's Hospital questionnaire in victims of the Tokyo subway sarin attack:

Frequency and percentage of symptoms in St. Luke's Hospital sarin patients
<table><tr><th>Symptom</th><th>1997(No. and % of 283 patients)</th><th>1998 (No. and % of 206 patients)</th><th>2000 (No. and % of 191 patients)</th></tr><tr><td>Tiredness of eyes</td><td>106 (37.5)</td><td>69 (33.5)</td><td>75 (39.3)</td></tr><tr><td>Dim vision</td><td>66 (23.3)</td><td>53 (25.7)</td><td>48 (25.1)</td></tr><tr><td>Difficulty focusing</td><td>60 (21.2)</td><td>36 (17.5)</td><td>34 (17.8)</td></tr></table>
The frequency of occurrence of the above symptoms was not found to be significant at the p<0.05 level. The 3 eye symptoms were some of the most common physical symptoms still reported after 5 years after the attack. Adding physical symptoms to the diagnostic criteria used for PTSD in the study contributed to make the diagnosis of PTSD more frequent</td><td><u>Strengths</u>
The study follows up a group of patients for 2, 3 and 5 years after the sarin attack

<u>Limitations</u>
The study is largely descriptive, with limited statistical analysis of the results;
questionnaire used was developed by St. Luke's Hospital, and is thus an unpublished, non peer-reviewed instrument;
no possible confounding factors are examined</td><td>The frequency with which the indicated symptoms were experienced by victims of the Tokyo subway incident 5 years after the event was not found to be at a significant level</td></tr>
<tr><td>Nakajima</td><td>1999</td><td>STUDY OF VICTIMS OF THE INCIDENT OF SARIN RELEASE IN A RESIDENTIAL DISTRICT OF MATSUMOTO IN JAPAN IN 1994

EXPOSURE 12 litres of sarin were released in a residential area of Matsumoto City, but individual exposure, or proximity of individuals to the release, were not measured or estimated; 93 persons had cholinesterase activity measured 4 weeks after the sarin exposure and again for the 1 year survey

The relationship between cholinesterase activity four weeks after sarin exposure and 2 neurological symptoms 1 year after the sarin incident in 93 subjects is given in the table below:</td><td><u>Strengths</u>
93 of the subjects had cholinesterase activity records from 4 weeks after the incident which could be considered for any association with symptoms at 1 year after the event</td><td>There is insufficient evidence from the study to suggest that the symptoms of fatigue and headache were significant health outcomes for victims of the Matsumoto sarin attack, or that they</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>

Cholinesterase activity 4 weeks after sarin exposure and symptoms 1 year after the event in 93 subjects

<table>
<tr><th>Symptoms</th><th>Number of subjects</th><th>Erythrocyte-Anticholinesterase (IU/L)</th><th>Serum-Anticholinesterase (IU/L)</th></tr>
<tr><td>Symptoms (any type of symptoms)</td><td>Yes 20
No 73</td><td>1.38±0.42•
1.56±0.29</td><td>5.57±0.77
5.48±0.93</td></tr>
<tr><td>Fatigue</td><td>Yes 18
No 75</td><td>1.29±0.39***
1.58±0.29</td><td>5.57±0.77
5.48±0.93</td></tr>
<tr><td>Headache</td><td>Yes 1
No 92</td><td>1.9
1.52±0.33</td><td>7.0
5.45±0.92</td></tr>
</table>

Notes: subjects are divided into those who experienced symptoms and those who did not; the numbers given represent the mean ±SD, or the values when the numbers were 3 or less;
* ,**,*** significant differences were noted between subjects who had and did not have symptoms at p<0.05, 0.01 and 0.001, respectively

From the table it can be seen that, of the 93 subjects with AChE measurements, those who had any type of symptoms 1 year after the event had had lower erythrocyte AChE activity 4 weeks after the event; also, those who had fatigue 1 year after the event had had significantly lower activity of erythrocyte AChE. The findings for headache are not significant, and only one subject reported this symptom.

HEALTH EFFECTS The neurological symptoms not described elsewhere in this review are those of fatigue and headache.
The prevalence of these symptoms described subjectively 1 year after sarin exposure in the Matsumoto incident is given in the table below:

Prevalence of symptoms 1 year after sarin exposure in Matsumoto incident

<table>
<tr><th>Symptoms</th><th>Admitted patients</th><th>Out patients</th><th>Non-patients</th><th>Total</th></tr>
<tr><td>Fatigue</td><td>11(100)*</td><td>17 (65.4)</td><td>7 (41.2)</td><td>35 (64.8)***</td></tr>
</table>

</td>
<td>The study follows some subjects for up to 3 years after the event

<u>Limitations</u>
The symptoms of fatigue and headache are self-reported and self-assessed

The background prevalence of these symptoms is unclear

The reporting of results for “All victims” and “Victims A” at 3 years after the event is confusing, and the symptoms may not be clearly linked to the sarin exposure

There is limited statistical analysis of the results</td>
<td>were clearly sequelae of the incident. Results after 3 years do not suggest significance</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Headache</td><td>0 (0)</td><td>2 (7.7)</td><td>1 (5.9)</td><td>3 (5.6)</td></tr>
</table>
Notes: Admitted patients are victims who required hospitalisation;
Out patients were victims who consulted doctors but did not require hospitalisation;
Non-patients were those who had symptoms but did not consult a doctor
Numbers of subjects are reported in the table, with percentages in brackets
* ,**,*** significant differences were noted between subjects who had and did not have symptoms at p<0.05, 0.01 and 0.001, respectively

Thus, at 1 year after exposure, there was significant prevalence of fatigue among persons who had been exposed.

The table below shows the relationship between sarin exposure and the neurological symptoms 3 years after the sarin incident:

Neurological symptoms 3 years after sarin exposure in Matsumoto incident
<table>
<tr><td>Symptoms</td><td>Non-victims 669</td><td>All victims 167</td><td>Odds Ratio (95% CI)</td><td>Victims A 167</td><td>Odds Ratio (95% CI)</td></tr>
<tr><td>Fatigue</td><td>22 (3.3)</td><td>25 (15.0)***</td><td>5.18 (2.84-9.44)</td><td>8 (4.8)</td><td>1.48 (0.65—3.37)</td></tr>
<tr><td>Headache</td><td>7(1.0)</td><td>14 (8.4)**</td><td>8.65 (3.43-21.81)</td><td>1 (0.6)</td><td>0.57 (0.07-4.54)</td></tr>
</table>
Notes: “Non-victims” are subjects who did not have one or more symptoms immediately after the sarin incident;
“All victims” are subjects who had one or more symptoms immediately after the sarin incident;
“ Victims A” are subjects who had one or more symptoms both 1 and 3 years after the sarin incident
* ,**,*** significant differences were noted between subjects who had and did not have symptoms at p<0.05, 0.01 and 0.001, respectively

A significant difference between victims and non-victims is noted for fatigue and headache immediately after the sarin incident; however, for cohort members who continued to experience symptoms both 1 and 3 years after the event, the CIs do not suggest significance for fatigue, and the OR does not suggest significance for headache
</td><td></td><td></td></tr>
<tr><td colspan="5">7.A.3. Cross-sectional studies</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td>McCauley</td>
<td>2001</td>
<td>
STUDY OF GULF WAR VETERANS POSSIBLY EXPOSED TO SARIN AT KHAMISIYAH, IRAQ

EXPOSURE Exposure to sarin was assumed for troops within a 50 km radius of the detonated chemical warfare agents at Khamisiyah in Iraq

HEALTH EFFECTS Odds Ratios and 95% CIs for symptoms reported by subjects approximately 9 years after the detonation of the munitions dump at Khamisiyah in Iraq, and not reported elsewhere in our summaries, are shown in the table below:

Neurological symptoms reported by Gulf War veterans 9 years after destruction of munitions dump at Khamisiyah
<table>
<tr><th></th><th>Deployed (n=1,263) vs. Non-deployed (n=516)

OR (95% CI)</th><th>Khamisiyah (n=653) vs. Non-Khamisiyah (n=610)

OR (95% CI)</th><th>Khamisiyah Witness (n=162) vs. Non-Khamisiyah Witness (n=405)
OR (95% CI)</th></tr>
<tr><td>Persistent fatigue, tiredness or weakness</td><td>6.2 (4.7, 8.4)</td><td>1.0 (0.7, 1.3)</td><td>1.8 (1.2, 2.6)</td></tr>
<tr><td>Increased sensitivity to everyday chemicals</td><td>3.1 (2.2, 4.3)</td><td>1.2 (0.8, 1.6)</td><td>1.5 (1.0, 2.3)</td></tr>
<tr><td>Continuous eye irritation or sensitivity</td><td>3.5 (2.4, 5.1)</td><td>1.1 (0.7, 1.5)</td><td>1.2 (0.8, 1.8)</td></tr>
<tr><td>Unexplained weight loss >10lb</td><td>3.5 (2.2, 6.0)</td><td>0.7 (0.4, 1.0)</td><td>1.9 (1.0, 3.3)</td></tr>
<tr><td>Unexplained weight gain >10lb</td><td>2.9 (2.2, 3.9)</td><td>1.0 (0.8, 1.4)</td><td>1.5 (1.0, 2.2)</td></tr>
</table>
Notes: “Deployed” were personnel deployed within a 50km radius of Khamisiyah, as well as personnel deployed in the Gulf but outside the 50km radius; these results are adjusted for age, gender, and region of residence
“Non-deployed” were personnel who had been on active duty at the time of the Gulf War, but not deployed to the Gulf
The “Khamisiyah vs. Non-Khamisiyah” results are adjusted for age and gender “Khamisiyah” were personnel thought to be operating within a 50km radius of the munitions dump;;
The Khamisiyah personnel are further divided into “Khamisiyah Witness”, a subgroup who had been involved in or watched the Khamisiyah detonation (n=162), and “Non-Khamisiyah Witness” (n=405) who had not been involved; the remaining 86 individuals were unsure which subgroup they belonged to
</td>
<td>
<u>Strengths</u>
Relatively large number of subjects took part in the study

<u>Limitations</u>
No direct measures of exposure;

Several possible sources of bias:
1) selection bias possible because sample limited to persons whose telephone numbers could be tracked down by common search mechanisms;
2) the Khamisiyah population of veterans was not evenly distributed throughout the US, the majority having North Carolina or Georgia addresses, so there may be bias due to location;
3) also possible misclassification, because 50 subjects considered by the Department of Defense to be in the Khamisiyah-deployed group reported they had never been in Iraq;
4) recall bias
</td>
<td>Due to the limitations of the study, in particular the difficulty of establishing precise location of personnel, the evidence provided is not conclusive that subjects directly involved in the detonation of chemical warfare agents containing sarin and cyclosarin, or located close to the site of the detonation, were more significantly affected than other personnel deployed in the Gulf area; significant associations relate more clearly to Gulf War veterans compared to veterans not deployed to the Gulf</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>Results appear to indicate significant Odds Ratios for Deployed versus Non-Deployed subjects; however, in the Khamisiyah versus Non-Khamisiyah analysis, there do not appear to be any significant associations. When the Khamisiyah subjects are subdivided into Witness and Non-Witness, there appear to be some significant associations again, suggesting that those who were directly involved in the detonation or may have been closest to the site may have suffered more symptoms. However, the precise location of personnel is in some doubt, as the authors report that 50 subjects considered by the Department of Defense to be in the Khamisiyah-deployed group reported that they had never been in Iraq. Furthermore, 86 subjects among the Khamisiyah personnel were unsure whether they were “Khamisiyah witness” or “Non-Khamisiyah witness”. Due to the difficulty of precisely establishing exposure of individuals, the results cannot be seen as conclusive evidence of an association between presumed exposure and symptoms in the subjects. Co-exposure to other chemicals was also possible</td><td>because the data collection took place 8-9 years after the events</td><td></td></tr>
<tr><td>McCauley</td><td>2002</td><td>STUDY OF GULF WAR VETERANS POTENTIALLY EXPOSED TO SARIN AT KHAMISIYAH, IRAQ

EXPOSURE Exposure to sarin was assumed for troops within a 50 km radius of the detonated chemical warfare agents at Khamisiyah in Iraq

HEALTH EFFECTS Frequency of diagnosed medical conditions reported by Gulf War Veterans and 95% likelihood-based CIs for neurological symptoms not discussed elsewhere are shown in the table below:

Frequency of diagnosed medical conditions reported by Gulf War veterans approximately 9 years after Khamisiyah incident
<table>
<tr><th rowspan="2">Symptom</th><th colspan="3">Frequency (%)</th><th colspan="2">OR (95% CI)</th></tr>
<tr><th>ND n=516</th><th>KHAM n=653</th><th>NKHAM n=610</th><th>Deployed vs ND</th><th>KHAM vs NKHAM</th></tr>
<tr><td>Epilepsy or seizures</td><td>0.2</td><td>0.4</td><td>0.5</td><td>2.7(0.4-51.3)</td><td>0.5(0.1-4.8)</td></tr>
<tr><td>Other neurological disease</td><td>1.4</td><td>2.2</td><td>2.5</td><td>1.8(0.8-4.5)</td><td>0.6(0.2-1.6)</td></tr>
</table>
Notes: KHAM – Khamisiyah veteran; NKHAM – non-Khamisiyah veteran; ND – veteran not deployed to the Gulf
The results suggest that veterans potentially exposed to low levels of sarin/cyclosarin in the vicinity of Khamisiyah did not differ from other veterans in the Gulf not deployed to Khamisiyah on the above self-reported neurological symptoms (KHAM vs. NKHAM). Some increased ORs were observed in the comparison of outcomes among veterans deployed to</td><td>Strengths and Limitations as above for McCauley 2001 study</td><td>The findings of the study relating to symptoms of epilepsy, seizures or other neurological disease not covered elsewhere in this review do not appear to be associated with potential exposure to sarin/cyclosarin at Khamisiyah; increased ORs relate to Gulf War veterans when compared to veterans who were not deployed to the Gulf, but are not statistically significant</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>the Gulf and other veterans who were not in the Gulf War (Deployed vs. ND), but CIs do not suggest significance</td><td></td><td></td></tr>
<tr><td>Ohtani</td><td>2004</td><td>STUDY OF PTSD SYMPTOMS IN 34 VICTIMS OF THE TOKYO SUBWAY SARIN ATTACK 5 YEARS AFTER THE INCIDENT
EXPOSURE Quantity of sarin not known; subjects had been treated in emergency wards after the attack

HEALTH EFFECTS Using the self-rating questionnaire developed by staff at St. Luke’s International Hospital, the following neurological symptoms were found most frequently in subjects:
blurred vision reported by 63.3% of subjects;
difficulty in focusing reported by 60%;
eyes tend to become easily tired, 56.7%;
difficulty in seeing far, 46.7%;
headache 44.8%;
easily fatigued 41.4%.
The eye symptoms appeared to be the most prominent</td><td><u>Strengths</u>
The focus of the study is PTSD, and a few neurological symptoms are reported alongside the psychological symptoms

<u>Limitations</u>
Small group of subjects

No analysis made of the reported neurological symptoms</td><td>It is not possible to make any conclusions about the neurological symptoms reported in this study, the focus of which is PTSD</td></tr>
<tr><td>Stallones</td><td>2002b</td><td>US STUDY OF DEPRESSIVE SYMPTOMS IN FARM RESIDENTS WITH AGRICULTURAL EXPOSURE TO PESTICIDES, INCLUDING OPs

EXPOSURE Self-reported exposure to pesticides on questionnaire; pesticides used on the farm included the OPs terbufos, chlorpyrifos, phosmet, dichlorvos, as well as other classes of pesticides.

HEALTH EFFECTS The study estimated ORs from conditional logistic regression analyses for neurological symptoms in 69 cases of acute pesticide-related illness and 692 farm residents not reporting a pesticide-related illness. The authors report that if a respondent used crop OPs,rather than any other pesticide, there were increased odds of having a pesticide-related illness of: OR=2.45, 95% CI 1.29-4.64.

The table below shows neurological symptoms not reported in other sections that were investigated for association with having had a pesticide-related illness:

Neurological symptoms associated with having had a pesticide-related illness
<table><tr><th>Neurological symptom</th><th>Odds Ratio</th><th>95% CI</th><th>χ^2 for trend</th><th>P value</th></tr></table></td><td><u>Strengths</u>
Relatively large study population of 761 respondents

<u>Limitations</u>
Exposure is self-reported

The regression analyses relate to pesticide-related illness, not specifically OP-related illness, although the use of crop OPs is a significant factor in assessing pesticide-related illness; co-exposure to</td><td>There is insufficient direct evidence of a specific acute exposure to OPs and an association with some neurological symptoms</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Tired more easily than expected</td><td>1.26</td><td>0.75-2.12</td><td>_</td><td>_</td></tr>
<tr><td>Had difficulty concentrating</td><td>2.07</td><td>1.22-3.50</td><td>9.20</td><td>0.0024</td></tr>
<tr><td>Been confused or disoriented</td><td>2.09</td><td>0.91-4.82</td><td>6.10</td><td>0.0135</td></tr>
<tr><td>Had trouble remembering things</td><td>1.34</td><td>0.80-2.25</td><td>9.27</td><td>0.0023</td></tr>
<tr><td>Had to make notes to remember things</td><td>2.18</td><td>1.20-3.97</td><td>10.54</td><td>0.0012</td></tr>
<tr><td>Found it hard to understand meaning of newspapers and books</td><td>1.90</td><td>1.01-3.60</td><td>6.35</td><td>0.0117</td></tr>
<tr><td>Relatives noticed that subject has trouble remembering things</td><td>2.54</td><td>1.47-4.39</td><td>22.40</td><td><0.0001</td></tr>
</table>
Four of these symptoms appear to be significantly associated with reporting a pesticide-related illness, based on the ORs and 95% CIs, and the χ^2 test for trend is highly significant for trouble remembering things, as noticed by relatives; OPs applied to crops were found to be associated with reporting a pesticide-related illness; thus the association with OPs is indirect
</td><td>pesticides other than OPs is likely</td><td></td></tr>
<tr><td colspan="5">B. Chronic Low Level Exposure</td></tr>
<tr><td colspan="5">7.B.1. Cohort studies</td></tr>
<tr><td>Albers</td><td>2004b</td><td>STUDY OF EFFECTS OF EXPOSURE TO CHLORPYRIFOS IN CHLORPYRIFOS PRODUCTION WORKERS

EXPOSURE Urinary excretion of OP metabolites was measured: excretion of 3,5,6 trichloro-2-pyridinol (TCP) was significantly higher in chlorpyrifos-exposed subjects (192.2 µg/g creatinine vs 6.2 µg/g creatinine in referents) (P<0.0001). Plasma butyrylcholinesterase (BuChE activity) was measured, and was significantly lower in chlorpyrifos-exposed subjects (7155 vs 8183 mu/ml; P<0.01). The ratio of lowest BuChE during follow-up period to baseline was significantly lower in chlorpyrifos exposed subjects.

HEALTH EFFECTS
Only 2 neurological symptoms, asterixis (tremor of wrist) and incoordination, are not covered in other sections of this review. The findings are that none of the subjects, either chlorpyrifos-exposed or referents, fulfilled clinical criteria for either of these symptoms</td><td><u>Strengths</u>
Biomonitoring measures are available for subjects and referents in the study

<u>Limitations</u>
These are not key symptoms considered in the study</td><td>There is no evidence of an association between exposure to chlorpyrifos and symptoms of wrist tremor or incoordination in this study</td></tr>
<tr><td colspan="5">7.B.3. Cross-sectional studies</td></tr>
<tr><td>Bazylewicz-Walczak</td><td>1999</td><td>STUDY OF BEHAVIOURAL EFFECTS OF OCCUPATIONAL EXPOSURE TO OPs IN POLISH FEMALE GREENHOUSE WORKERS</td><td><u>Strengths</u>
Monitoring: air levels of OPs recorded in</td><td>The results of the study suggest that greenhouse workers</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>EXPOSURE Data for 26 Polish women working in greenhouses was reported as total OPs (combined dermal/respiratory exposure) on days 1-6 after the restriction period, a period of 1-3 days after spraying of pesticides when workers may not enter the sprayed area. Most frequently used compounds were dichlorvos, methamidophos, methidathion and pirimiphos-methyl. Workers had additional exposures to carbamates, synthetic pyrethroids and dithiocarbamates Exposure was seasonal, each worker being in contact with OPs for approximately 7-46 days. There were cycles of 4-7 days of spraying with an interval of approximately 3 days between spray applications. The daily cumulative exposure (combined inhalation/dermal) on 6 consecutive days after the restriction period was between 0.001-0.009 % of the toxic dose (based on dermal LD50s). The duration of spraying varied between 1-24 years (mean 11.9y SD 6.8y).

Concentrations of OP pesticides in the air in greenhouses did not exceed acceptable limits; exposure level of workers measured from levels on clothing and skin was found to be low, below 0.010% of the toxic dose based on acute dermal LD50.

HEALTH EFFECTS Neurological symptoms not covered in the Peripheral Neuropathy section are grouped as “Neurological symptoms – central” in the paper. These symptoms refer to questions in the authors’ Subjective Symptoms Questionnaire relating to vertigo, trembling of the hands, dropping objects easily from the hands, finding it difficult to walk in the dark, experiencing changes in sense of smell or taste, and experiencing numbness or strange sensations in the muscles of the face. Two-way ANOVA was performed , and mean values and standard deviations in the number of symptoms experienced are reported for a pre-season examination (January- February), before intensive spraying in the greenhouses began, and for a second post-season examination (June), conducted in the period following major spraying operations. The significance level α was taken to be 0.10:

<table>
<tr><th>Name of test variable</th><th colspan="2">Factor “season”
F p</th><th colspan="2">Factor “group”
F p</th><th colspan="2">Interaction
F p</th></tr>
<tr><td>Subjective Symptoms Questionnaire</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Neurological symptoms - central</td><td>4.58</td><td>.04</td><td>4.26</td><td>0.04</td><td colspan="2">NS</td></tr>
</table>

Notes: NS=not significant
Factor “season”= single spraying season effects
Factor “group” = long-term effects of exposure
Interaction= interaction between short- and long-term effects of exposure

In the category “Factor ‘season’”, results obtained in the pre-season and post-season examination were compared for exposed workers. The significant p values are for improvement in terms of the number of symptoms reported. Thus the spraying season was</td>
<td>greenhouses, and exposure of workers measured by analysis of levels on clothing and skin

<u>Limitations</u>
Small sample size

The results are analyses done in a single year, not observations over a longer span of time</td>
<td>seasonally exposed to non-toxic levels of OPs did not suffer detrimental effects after one season of spraying. The comparison with non-exposed controls suggests there were more neurological symptoms reported in exposed women than in controls, but the interaction of short- and long-term effects was not significant. The small number of subjects is a limitation of the study</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>not seen to have a detrimental effect.
In “Factor ‘group’”, the exposed and control groups were compared for possible long-term effects of exposure. Exposed women reported more neurological symptoms than controls, but no significant interaction effects were seen between short- and long-term effects of exposure</td><td></td><td></td></tr>
<tr><td>Cox</td><td>2005</td><td>EVALUATION OF POTENTIAL HEALTH EFFECTS ON RESIDENTS OF SPRAYING OF METHYL PARATHION IN THE HOME

EXPOSURE Authors report that no difference in average acetylcholinesterase levels was observed between low- and high-exposure groups

HEALTH EFFECTS Authors report no significant differences in any of the health outcome measurements between the exposure group (homes containing >150μg/100cm²) and the control group (homes containing <15 μg/100cm²). However, some differences in health outcome measures were seen at high exposure levels, that is, where average levels of methyl parathion in the home were >260μg/100cm²; the table below indicates those neurological symptoms that were reported with increased frequency by subjects whose home contained >260μg/100cm² of methyl parathion:

<table><tr><th>Symptom</th><th>Frequency* <260μg/100cm²</th><th>Frequency* >260μg/100cm²</th><th>P</th><th>OR(95% CI)</th></tr><tr><td>n</td><td>231</td><td>60</td><td></td><td></td></tr><tr><td>Weakness</td><td>14%</td><td>26%</td><td>0.03</td><td>2.1(1.1-4.3)</td></tr><tr><td>Headache</td><td>20%</td><td>33%</td><td>0.05</td><td>1.9(1.0-3.7)</td></tr><tr><td>Lack of energy</td><td>14%</td><td>28%</td><td>0.02</td><td>2.4(1.2-4.9)</td></tr><tr><td>Memory problems</td><td>8%</td><td>16%</td><td>0.08</td><td>1.7(0.9-5.0)</td></tr></table>
* the percentage of residents who reported the symptoms

Logistic regression analysis also demonstrated an OR of 2.0 for the presence of possible subacute toxicity for a unit increase of home methyl parathion of 200 μg/100cm² above the median value of 260μg/100cm² (subacute toxicity defined as 2 or more neurologic symptoms in 2 or more systems, lasting >10 days or recurrent episodes, and temporally related to spraying of methyl parathion)

Overall, symptoms of weakness, headache and lack of energy appear to be significantly associated with high levels of methyl parathion, >260μg/100cm², sprayed in the home</td><td><u>Strengths</u>
Measurement of levels of methyl parathion in homes

Medical evaluations were performed by physicians

<u>Limitations</u>
Authors recognise that the exposure estimate is at best only semi-quantitative, since methyl parathion levels were averaged over several rooms in the house
Many high samples occurred under kitchen sink and in cabinets, areas to which not all family members would be exposed

No attempt made to measure potential exposures to methyl parathion or other OPs outside the home;

Subjects not blinded</td><td>Individuals living in homes with high levels of methyl parathion may have an increased incidence of some chronic neurological symptoms; however, since average acetylcholinesterase (AChE) levels were not found to be lower in exposed individuals, the reported symptoms do not appear to be related to AChE depression</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td></td><td>to the methyl parathion in their homes, so possible recall bias</td><td></td></tr>
<tr><td>Kamel</td><td>2005</td><td>STUDY OF NEUROLOGICAL SYMPTOMS IN U.S. PRIVATE PESTICIDE APPLICATORS IN THE AGRICULTURAL HEALTH STUDY

EXPOSURE Self-reported exposure; cumulative use of OPs (lifetime days, without or with use in past year) was calculated. Cumulative use also calculated for other chemical classes, including organochlorines, carbamates, pyrethoids

HEALTH EFFECTS In models including cumulative use, applicators in highest category of OP use (>500 lifetime days) were 1.6-1.7 times more likely to experience ≥10 symptoms in year before enrolment, and averaged 1.0-1.1 additional symptoms, compared with lowest quartile.

Associations of some specific neurological symptoms with OP exposure among licensed pesticide applicators enrolled in the Agricultural Health Study 1993-1997 (n=18,782) are shown in the table below:

<table><tr><th>Symptom</th><th>Exposure to Organophosphates (OR for experiencing symptom with high frequency compared to low frequency)</th></tr><tr><td>Headache</td><td>1.62*</td></tr><tr><td>Loss of appetite</td><td>1.76*</td></tr><tr><td>Poor night vision</td><td>1.85*</td></tr><tr><td>Blurred/double vision</td><td>1.87*</td></tr><tr><td>Changes in smell or taste</td><td>1.83*</td></tr><tr><td>Difficulty speaking</td><td>1.94*</td></tr></table>
Notes: estimates are for the highest category of lifetime days of use, >500 days
* estimates for which the 95% Confidence Interval excludes 1.00

Overall, the authors found dose-related associations of symptom count to cumulative exposure to OPs, whether or not individuals with history of pesticide poisoning or a high-exposure event were included, suggesting that moderate (non-acute) exposure itself is</td><td><u>Strengths</u>
Large study size, because a cross-sectional analysis is made of data from a large cohort study (Agricultural Health Study)

<u>Limitations</u>
Only 44% of private applicators completed the relevant questionnaire, so potential bias is a concern

All symptoms, as well as exposure, are self-reported, raising concern of potential recall or reporting bias

Likely co-exposure of participants to other pesticides</td><td>The study results suggest a possible association of some neurological symptoms with cumulative lifetime exposure to OPs, although neurological examination was not performed, and co-exposure to other pesticides besides OPs may be a confounding factor</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>associated with increased risk</td><td></td><td></td></tr>
<tr><td>Kilburn</td><td>1999</td><td>U.S. STUDY OF EFFECTS OF CHLORPYRIFOS SPRAYED INDOORS ON 22 SUBJECTS

EXPOSURE Schedules of pesticide application were found for offices of 6 patients, so concentrations of applied chlorpyrifos were known; 4 patients had personal clothing or rugs analysed for chlorpyrifos; for other subjects, self-reported exposure on questionnaire

HEALTH EFFECTS
The table below indicates the frequency of neurological symptoms not covered in other sections, that were included in the questionnaire used in this study

<table>
<tr><th></th><th>Exposed (mean±SD) n=22</th><th>Unexposed (mean±SD) n=264</th><th>p</th></tr>
<tr><td>Decreased smell</td><td>4.5±3.1</td><td>2.1±1.9</td><td>0.0001</td></tr>
<tr><td>Headache</td><td>7.7±3.1</td><td>4.1±2.7</td><td>0.0001</td></tr>
<tr><td>Chest tightness</td><td>5.5±3.0</td><td>2.2±1.8</td><td>0.0001</td></tr>
<tr><td>Palpitations</td><td>5.6±3.2</td><td>2.1±1.8</td><td>0.0001</td></tr>
<tr><td>Burning in chest</td><td>4.2±2.5</td><td>2.0±1.7</td><td>0.0001</td></tr>
<tr><td>Shortness of breath</td><td>4.5±2.7</td><td>2.5±2.0</td><td>0.0001</td></tr>
</table>
There appears to be a significantly increased frequency of these symptoms in exposed subjects compared to unexposed, but exact exposure is not known, and differs in nature between the participants. Some of the subjects were also exposed to other chemicals</td><td><u>Strengths</u>
Objective measures of exposure were available for 10 of the 22 exposed participants

<u>Limitations</u>
Small study size

The questionnaire relating to symptom frequencies was devised by the group and is not a validated instrument

The actual dose of chlorpyrifos received is uncertain, and amoounts of chlorpyrifos in the air are not known.

Some of the subjects may have had co-exposure to other pesticides</td><td>The neurological symptoms reported here appear to be significantly increased in exposed subjects, but the study only includes 22 individuals, and not all were exposed in the same circumstances. Actual dose and exposure to chlorpyrifos is unknown</td></tr>
<tr><td>Lee</td><td>2003</td><td>SOUTH AFRICAN STUDY OF ASSOCIATION BETWEEN PARAOXONASE GENE POLYMORPHISM AND CHRONIC SYMPTOMS IN PESTICIDE-EXPOSED WORKERS

EXPOSURE Self-reported pesticide applicator history, medical history and symptoms. Although this paper refers only to “pesticide”, not specifically OP, exposure, authors refer to a previous paper (London et al, Effects of long-term organophosphate exposures on neurological symptoms, vibration sense and tremor among South African farm workers, Scand J Work Environ Health1998, 24: 18-29) which indicates that exposure assessment was based on an OP job exposure matrix.

HEALTH EFFECTS The authors state that they considered the following symptoms to be</td><td><u>Strengths</u>
The study contains measures of serum albumin, gamma glutamyl transferase, and cholinesterase plasma and red blood cell levels, and the levels are compared between the two groups of</td><td>The measure of neurological symptoms and definition of chronic toxicity in this study is too imprecise to draw conclusions</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>compatible with chronic toxicity: chronic abdominal pain, nausea, rhinorrhea, dizziness, headache, somnolence, fatigue, gait disturbance, limb numbness, paresthesias, limb pain or limb weakness. The symptoms are not reported on separately, but put together to give a “Neurologic symptom score”, which is subdivided into 2 sections, subjects with a score of ≥2 symptoms being categorised as having toxic symptoms, and subjects with a score of ≤1 as not having toxic symptoms:

<table><tr><th>Variable</th><th>≤1 Toxic symptoms Mean value (SD)</th><th>≥2 Toxic symptoms Mean value (SD)</th></tr><tr><td>Neurologic symptom score</td><td>0.4 (0.5)</td><td>4.1 (2.3)</td></tr></table>
There is a P value of 0.0001 for the Neurologic symptom score being significantly higher in subjects with more than 2 of the symptoms considered, and subjects with 1 or 0 symptoms. This is the only analysis made of the neurological symptoms, and the focus of the study is the interaction of genotype and pesticide exposure</td><td>subjects

<u>Limitations</u>
The measure of neurological symptoms is imprecise, since the numerical score can include very different symptoms

The 100 subjects chosen for genotyping were selected by the authors “by convenience”, so there is potential for bias in this selection</td><td></td></tr>
<tr><td>Solomon</td><td>2007</td><td>UK STUDY OF NEUROPSYCHIATRIC SYMPTOMS IN PAST USERS OF SHEEP DIP AND OTHER PESTICIDES

EXPOSURE Self-reported on questionnaire, lifetime history of work with the following five categories of pesticides: sheep dip, other insecticides, herbicides, fungicides and wood preservatives

HEALTH EFFECTS Study examines whether there is increased prevalence and unusual clustering of postulated COPIND (Chronic OrganoPhosphate-Induced Neuropsychiatric Disorder) symptoms, a particular grouping of neurological and psychiatric symptoms. Psychiatric symptoms are described in the Psychiatric outcome section. This section describes the neurological symptoms considered.

Prevalence of 7 neurological symptoms analysed according to previous work with pesticides. The 7 symptoms were:-
difficulty concentrating; difficulty remembering things; difficulty with handwriting; difficulty speaking; sensitivity to certain smells; increased sensitivity to effects of alcohol; tiredness and lack of energy.

1) Prevalence of neurological symptoms according to previous work with pesticides
Prevalence is reported as the percentage of men reporting a symptom. In comparison with</td><td><u>Strengths</u>
Large number of subjects, despite low response rate of only 31%

Many adjustments made for possible confounding factors

<u>Limitations</u>
Possible selection bias, given the low response rate, with over-representation of subjects with symptoms in the study sample

The ‘somatising tendency’ score is a</td><td>Seven neurological symptoms investigated in the study were consistently more common in past users of sheep dip than in men who had never used pesticides, but the association was not specific to sheep dip or insecticides, and was also observed with other pesticides. Clustering of symptoms was observed, but was not specific to sheep dip or</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
men who had never worked with pesticides, those with occupational exposure to sheep dip had a higher prevalence of each symptom, with the largest relative excess being for sensitivity to smells (7.1% vs. 4.5%). However, symptoms were also more frequent in those who had worked with other pesticides but not with sheep dip.

2) Clustering of neurological symptoms according to previous work with pesticides
Analysis made of extent to which the 7 neurological symptoms clustered within individuals. Overall, strong evidence of clustering, but no evidence that clustering was specific to work with sheep dip or insecticides. No indication of a different pattern of symptom clustering among men who had worked with sheep dip compared with those who had worked only with other pesticides, or not with pesticides at all: e.g. OR=18.4 for association between difficulty speaking and difficulty with handwriting for men who worked only with pesticides other than sheep dip or insecticides, and OR=21.2 for men who never worked with any pesticides.

3) Associations of neurological symptoms with occupational exposure to pesticides and somatising tendency
<table>
<tr><th>Risk Factor</th><th>All subjects n</th><th>≥3 Neurological symptoms* n</th><th>PR (Prevalence Ratio) (95% CI)∞</th><th>PR (95% CI)§</th><th>≥4 Neurological symptoms * n</th><th>PR (95% CI)∞</th><th>PR (95% CI)§</th></tr>
<tr><td>Occupational exposure to pesticides</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Never worked with pesticides</td><td>6109</td><td>243</td><td>1.0</td><td>1.0</td><td>111</td><td>1.0</td><td>1.0</td></tr>
<tr><td>Ever worked with sheep dip</td><td>1913</td><td>111</td><td>1.5(1.2-1.9)</td><td>1.3(1.0-1.6)</td><td>58</td><td>1.7(1.2-2.4)</td><td>1.4(1.0-2.0)</td></tr>
<tr><td>Ever worked with insecticides, never with sheep dip</td><td>832</td><td>51</td><td>1.5(1.1-2.0)</td><td>1.4(1.0-1.8)</td><td>20</td><td>1.3(0.8-2.1)</td><td>1.2(0.7-1.9)</td></tr>
<tr><td>Ever worked with other pesticides, never with sheep dip or insecticides</td><td>990</td><td>58</td><td>1.5(1.1-2.0)</td><td>1.3(1.0-1.7)</td><td>23</td><td>1.3(0.8-2.1)</td><td>1.1(0.7-1.7)</td></tr>
<tr><td>Somatising tendency score**</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>0</td><td>5263</td><td>87</td><td>1.0</td><td>1.0</td><td>38</td><td>1.0</td><td>1.0</td></tr>
</table>
</td>
<td>measure created by the study authors and is not a validated measure</td>
<td>insecticides, so evidence does not appear to support the presence of an OP-induced neurological disorder (COPIND)</td>
</tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions

0.1-0.5	2705	91	2.0(1.5-2.7)	2.0(1.5-2.7)	22	1.1(0.7-1.9)	1.1(0.7-1.9)
0.6-1.0	1214	136	6.8(5.2-8.9)	6.7(5.1-8.8)	60	6.9 (4.6-10.3)	6.8 (4.5-10.2)
>1.0	581	145	15.3 (11.7-20.0)	15.0 (11.4-19.5)	89	21.5 (14.7-31.6)	21.1 (14.4-30.9)

Notes: * for each outcome, the comparator for risk estimates was all subjects who did not have the outcome

∞ adjusted for age (in 8 bands) and area

§ adjusted for age and area, and also mutually adjusted

** "somatisation" means experiencing medically unexplained symptoms. The authors used responses to the Brief Symptom Inventory (BSI) to derive a "somatising tendency" score(81 of the 9844 subjects were excluded because somatising tendency score was missing).

For reporting multiple neurological symptoms, there was a strong relationship with somatising tendency. Thus, reporting 4 or more neurological symptoms was over 20 times more common in men with a somatising tendency score greater than 1, than in those with a somatising tendency score of 0.

Reporting of multiple neurological symptoms was more frequent among men who had worked with pesticides, even after adjusting for somatising tendency. No consistent differences were observed in associations with different classes of pesticide, but for reporting 4 or more neurological symptoms, the strongest association was with sheep dip (PR=1.4, 95% CI 1.0-2.0).

4) Associations with neurological symptoms among past users of sheep dip

Risk Factor	**All past users of sheep dip** n+	**≥3 Neurological symptoms*** n	PR (95% CI)∞	PR (95% CI)§	**≥4 Neurological symptoms*** n	PR (95% CI)∞	PR (95% CI)§
Lifetime use of sheep dip (days)							
<10	416	17	1.0	1.0	9	1.0	1.0
10-49	700	35	1.3(0.7-2.4)	1.3(0.7-2.4)	17	1.3(0.6-2.8)	1.3(0.6-2.9)
50+	693	54	2.1(1.2-	1.6(0.9-	28	2.1(1.0-	1.4(0.6-

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions

			3.6)	2.8)		4.6)	3.0)
Handled sheep dip concentrate							
No	544	31	1.0	1.0	14	1.0	1.0
Yes	1369	80	1.1(0.7-1.6)	1.0(0.6-1.5)	44	1.3(0.7-2.4)	1.1(0.6-2.1)
Ever experienced symptoms within 48h of work with sheep dip							
Never	1355	58	1.0	1.0	26	1.0	1.0
Fever or chills[¥]	101	14	3.9(2.1-7.1)	2.1(1.1-3.9)	7	4.9 (2.0-11.6)	1.9(0.8-5.0)
≥4 symptoms[¥]	156	31	5.1(3.2-7.9)	2.4(1.5-3.9)	17	6.8 (3.6-12.8)	2.7(1.4-5.5)

Notes: * for each outcome, the comparator for risk estimates was all men who did not have the outcome

∞ adjusted for age (in 8 bands) and area

§ adjusted for age and area, and somatising tendency

+ information on risk factors was missing for some subjects

[¥] these risk factors overlapped; each was analysed separately relative to never having experienced acute symptoms

The above table indicates that neurological symptoms were more common among men who reported having experienced multiple symptoms or fever/chills on at least one occasion within 48 hours of using sheep dip. Neurological symptoms were not associated with handling sheep dip concentrate. The prevalence of multiple neurological symptoms tended to be highest in those who had used sheep dip most often.

5) Lifetime prevalence for consulting a GP for 2 neurological symptoms, according to previous work with pesticides

Reason for consulting GP	Never worked with pesticides n Prevalence(%)*	Ever worked with sheep dip n Prevalence(%)*	Ever worked with insecticides, never with sheep dip n Prevalence(%)*	Ever worked with other pesticides, never with sheep dip or insecticides n Prevalence (%)*

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Difficulty speaking (e.g. in finding the right words or getting words out)</td><td>128 2.0</td><td>40 2.3</td><td>17 2.1</td><td>19 1.9</td></tr>
<tr><td>Difficulty with handwriting</td><td>98 1.5</td><td>32 1.9</td><td>10 1.3</td><td>14 1.5</td></tr>
</table>
Note: * prevalence rates are directly standardised to the age distribution of the total study sample

The table indicates that the lifetime prevalence for consulting a GP for the 2 categories of neurological symptoms described was marginally higher among men who worked with sheep dip than in those who had never worked with pesticides.

6) Conclusions
Overall, the 7 neurological symptoms tended to cluster in individuals, and were more common in past uses of sheep dip than in men who had never worked with pesticides, but the clustering was not specific to those who had worked with sheep dip. Reporting multiple neurological symptoms was more common in men who had worked with sheep dip, but the association was not specific to sheep dip, or to insecticides. There was also no relation to handling the concentrate, which is a known determinant of higher exposure.
</td><td></td><td></td></tr>
</table>

Table 8. EXPOSURE SCENARIO AND 'OTHER' NEUROLOGICAL EFFECTS

A. ACUTE EXPOSURE WITH CHRONIC EFFECTS

Table 8.A.1. Cohort Studies

Neurological Symptoms	First Author and Date	
	Kawana 2001	Nakajima 1999
Tiredness of eyes	-	
Dim vision	-	
Difficulty focusing		
Fatigue/tiredness/weakness		-
Headache	-	-

Table 8.A.3. Cross-Sectional Studies

Neurological Symptoms	First Author and Date			
	McCauley 2001	McCauley 2002	Ohtani 2004	Stallones 2002b
Fatigue/tiredness/weakness	+		+	-
Increased sensitivity to everyday chemicals	+			
Eye irritation	-			
Unexplained weight loss	-			
Unexplained weight gain	+			
Blurred vision			+	
Difficulty focusing			+	
Tiredness of eyes			+	
Headache			+	
Epilepsy or seizures		-		
Difficulty concentrating				+
Confused or disoriented				-
Trouble remembering things				-
Relatives notice that subject has trouble remembering things				+
Had to make notes to remember things				+
Difficulty understanding meaning of newspapers/books				+

B. CHRONIC LOW-LEVEL EXPOSURE

Table 8.B.1. Cohort Studies

Neurological Symptoms	First Author and Date
	Albers 2004b
Wrist tremor	-
Incoordination	-

Table 8.B.3. Cross-Sectional Studies

Neurological symptoms	First Author and Date					
	Bazylewicz-Walczak 1999	Cox 2005	Kamel 2005	Kilburn 1999	Lee 2003	Solomon 2007
Fatigue/tiredness/weakness		+				
Blurred vision			+			
Poor night vision			+			
Headache		+	+	+		
Memory problems		-				
Lack of energy		+				
Loss of appetite			+			
Changes in smell or taste			+	+		
Sensitivity to smells						+
Difficulty speaking			+			+
Difficulty with handwriting						+
Chest tightness				+		
Palpitations				+		
Burning in chest				+		
Shortness of breath				+		
Neurological symptom score for central nervous system symptoms	-				+	
Clustering of neurological symptoms						+

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF ALZHEIMER'S DISEASE AND DEMENTIA

OVERVIEW OF COT 1999 REPORT

1. The COT evaluation published in 1999 can be found in Chapter 7 of the report provided in Annex 16.

2. The COT 1999 report did not specifically consider Alzheimer's Disease (AD) or dementia. However, the COT noted in the executive summary of the report that further research was necessary to establish whether the risk of more severe neurological or neuropsychiatric diseases is increased by low-level exposure to OPs.

OVERVIEW OF LITERATURE PUBLISHED AFTER COT 1999 REPORT

Alzheimer's Disease / Dementia Symptoms and Risk Factors

3. As with all neurodegenerative diseases, Alzheimer's Disease (AD) is typically slowly progressive and associated with a variable gradual neurological dysfunction. AD is considered a form of dementia that gradually gets worse over time. The symptoms are similar to other dementias and results in cognitive deficits (e.g. memory, thinking, language, attention, perception) and behavioural disorders (e.g. wandering, agitation, yelling and persecutory ideation). The most severe symptoms include an inability to understand language, recognise family members, perform basic activities of daily living, incontinence and swallowing problems. Sufferers tend to become severely disabled and develop infections and organ failure. Early onset AD is associated with more progressive symptoms that appear before age of 60 years, while late onset AD is the most common type occurring in people aged 60 years and older.

4. Various diseases and medical conditions are known to be associated with dementia defined as a decline in cognitive function or mental ability (Table 9, Annex 12). These include a brain tumour, severe depression, stroke, various neurodegenerative diseases, as well as anaemia, and vitamin deficiency. Risk factors for AD include, age (although AD is not part of the normal aging process), family history, and susceptible genotype (APOE ϵ4 allele). Other possible contributing factors include gender, prolonged high blood pressure, and a history of head trauma.

Acute OP Exposure and AD / Dementia

5. For the purpose of this review, acute exposure is defined as a single incident where the exposure is continuous and may last up to a number of days, e.g. four days.

6. No study provided data on the chronic effects of acute exposure to OPs in relation to the potential development of AD or dementia.

Chronic OP Exposure and AD / Dementia

7. For the purpose of this review, chronic exposure is defined as a multiple repeat incident with a variable length of exposure (e.g. lasting for one season up to a lifetime).

8. One study evaluated the chronic effects of prolonged low-level exposure to OPs in relation to the potential development of AD, or dementia. This single cohort study examined OP exposure in residents of an agricultural community whose main occupation was farming (Hayden et al 2010).

9. Below is a summary of the key study details and findings. Further information can be found in Annex 12, which present information on the study design used (Table 6), and the results, strengths/weaknesses and conclusions (Table 7).

Cohort study

10. Hayden and colleagues (2010) examined the association of occupational pesticide exposure and the risk of incident dementia and AD in later life in residents of the US agricultural community of Cache County, Utah by assessing their cognitive status at baseline and after 3, 7 and 10 years (Table 6.B.1, Annex 12). Subjects aged 65 years or more as of January 1995 were taken from a well-characterised prospective cohort (Cache County Memory Study) to which 5092 residents completed baseline cognitive screening and risk factor questionnaires (90% response rate). However, at baseline the final eligible study participant group comprised of 3084 individuals who were without dementia and provided self-reported information about their pesticide exposure history. Reasons for non-participation included the subject dying or moving before the first follow-up, or refusal (n=486), which the authors did not further evaluate. However, the authors did conduct comparative analyses on subjects who were excluded on the grounds of ineligibility or providing insufficient information, and accounted for any differences in subsequent analyses. A total of 572 subjects reported being exposed to a range of pesticides (i.e. OPs, carbamates, organochlorines, and methyl bromides). No further information on duration and frequency of pesticide use was provided. The referent group comprised of 2512 subjects who reported not being exposed to any pesticides. Demographic differences between the two groups were accounted for in subsequent analyses. Standardised methods used to detect and diagnose dementia and AD included use of a version of the Modified Mini-Mental State Examination (3MS) and a dementia questionnaire, followed by a full clinical evaluation (using standard AD

diagnostic criteria) for individuals who either fell below a predefined cutpoint at screening or were of a certain age (90 years at baseline, or 85 years at subsequent follow-ups). The authors calculated Cox proportional hazard ratios to assess the risk from any or specific pesticide exposure, and adjusted for factors known to independently influence the risk of dementia and AD (i.e. age, education and APOE status); as well as for gender, and baseline 3MS scores. However, the authors did not account for the fact that most of the residents where followers of the Latter Day Saints movement which would impact on their lifestyle and subsequent health.

11. A total of 500 individuals were identified with incident dementia, with 344 having a primary or secondary diagnosis of AD (Hayden 2010, Table 7.B.1, Annex 12). The authors did not indicate the severity of symptoms. The risk of either health outcome was significantly increased in subjects exposed to any pesticide. However, after adjusting for potential confounding factors, the only significant finding associated with exposure to a specific pesticide group was observed for AD and organophosphates (HR=1.53[95% CI: 1.05-2.23]; p<0.03). The effect of co-exposure to organochlorines in 177 subjects did not have any significant effect on the risk of AD/dementia.

12. The findings of this reasonably designed large cohort study show that US farmworking residents of an agricultural community exposed to OP pesticides followed up for an average of seven years had a 1.5-fold increased risk of developing AD compared to unexposed residents. However, this finding should be considered with caution due to the study's use of a crude and subjective exposure assessment based only ever/never pesticide exposure, and the study's limited external validity given the fact subjects had religious affiliations that could potentially impact on their lifestyle and subsequent rates of chronic disease.

Summary

13. It is not possible to make any firm conclusions on the evidence reported from just one cohort study (Hayden et al 2010). Furthermore, despite the study's use of standard diagnostic criteria it should be noted that is generally difficult to distinguish AD from other forms of dementia, as clinical tests provide only approximately 85% certainty of an accurate AD diagnosis; the most definitive being pathological changes detected from brain tissue at autopsy.

14. The findings of the above study suggest that exposure to pesticides (e.g. OPs) may increase the risk of AD in later life. However, evidence from more studies would be required to sufficiently conclude on the significance of these findings. This updates and accords with the COT 1999 conclusions with regard to the need for more studies evaluating the risk of developing severe neurological diseases from prolonged low-level OP exposure.

Table 6. ALZHEIMER'S DISEASE AND DEMENTIA STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
B. Chronic low-level exposure									
6.B.1. Cohort studies									
Hayden	2010	US	Residents of the agricultural community of Cache County, Utah aged 65 years or more as of January 1995 taken from the Cache County Memory Study (a well-characterised prospective cohort). The sample population comprised of 5092 of all residents (90% of the area's population aged 65 years or more) who completed baseline cognitive screening and risk factor questionnaires. 359 subjects with prevalent dementia were excluded resulting in an eligible population of 4733. A further 1215 subjects did not participate in the first follow-up due to them either dying (586), moving (133) or refusing (496). The authors did not evaluate reasons for refusals to participate. A further 434 subjects were excluded on the	2512 Unexposed participants i.e. subjects who were not exposed to any pesticide. Comparative group analysis showed that subjects were similar with respect to baseline age and APOE status. Differences were apparent with regard to exposed subjects being male ($p<0.0001$), having more years of education ($p<0.01$) and a slightly lower baseline 3MS score ($p<0.05$).	Standardised methods were used for detection and diagnosis of dementia and AD. Cognitive status was assessed at baseline and at the first follow-up via use of a 2-stage screening tool i.e. a version of the Modified Mini-Mental State Examination (3MS) (that was adapted for epidemiological studies) and a Dementia Questionnaire. The authors subsequently noted that cognitive status was re-evaluated at each time point A full clinical evaluation for dementia and for milder clinical syndromes were performed for individuals who either fell below a predefined cutpoint at screening or were aged 90+ (at baseline) or aged 85+ (at subsequent follow-	Dementia and AD	Detailed occupational history questionnaires seeking information on work duration and pesticide exposures (e.g. type used, frequency and duration of use) were completed at baseline. Specific pesticides used included OPs, carbamates, organochlorines (DDT) and methyl bromides. For specific pesticide group analyses the authors specifically set aside/ excluded individuals who reported exposure to pesticides other than the group under study in order to clearly distinguish between the exposed and unexposed group	Age (at baseline), sex, education in years, *APOE* e4 status, baseline 3MS scores (to control for baseline cognitive status). Also, subjects were excluded if they had prevalent dementia at baseline, or provided missing data (i.e. on exposure, genotype, education and diagnosis). Excluded subjects tended to be older, female and less educated ($p<0.0001$) although their APOE status and pesticide exposure status were similar to those included in the study. NB. The authors also noted that the sample population were largely members of the Church of Jesus Christ of Latter-Day Saints, which influences the amount of alcohol and tobacco use and subsequent rate chronic disease.	Demographic characteristics of the exposed and unexposed members of the sample were compared using Chi-squared tests for categorical variables and t tests were used for continuous variables. Cox proportional hazards survival analysis was used to evaluate the risk of incident dementia and AD associated with pesticide exposure. Models were adjusted for factors known to independently influence the risk of dementia and AD (i.e. age, education and APOE status) as well as other factors

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			basis of not providing sufficient data which resulted in 3084 individuals at baseline without dementia providing self-reported information about their pesticide exposures. 572 subjects reported being exposed to pesticides of which Subjects were followed up at baseline and after 3, 7, and 10 years (average duration 7.2 years (SD, 3.5)		ups). No further information re: clinical evaluation was provided. Final diagnoses were assigned at consensus conferences using standard criteria: AD diagnosis was based on criteria developed by the *DSM-III-R* and the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association criteria; vascular dementia diagnosis was based on the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria. The authors defined dementia onset age as the year in which a participant unambiguously met *DSM-III-R* criteria for dementia				

Table 7. ALZHEIMER'S DISEASE AND DEMENTIA RESULTS AND CONCLUSIONS

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td colspan="5">B. Chronic Low-Level Exposure</td></tr>
<tr><td colspan="5">7.B.1. Cohort studies</td></tr>
<tr><td>Hayden</td><td>2010</td><td>[RESIDENTS OF A US AGRICULTURAL COMMUNITY EXPOSED TO PESTICIDES]

Exposure Data: More than 40% of exposed subjects reported farming as their primary occupation. Subjects did not provide adequate information on frequency or duration of pesticide use and therefore the authors classified participants as ever/never users in order to retain the largest possible sample for analysis. A total of 572 provided information about their pesticide exposure, to which 316 were exposed to OPs, 256 to organochlorines, 25 to carbamates and 28 to methyl bromides. 186 subjects reported having >1 type of exposure with 177 being exposed to both OPs and OCs and 164 having unclassifiable exposures. For specific pesticide group analyses a total of 256 individuals reporting exposures other than OPs were set aside in the analyses of OPs.

Health Outcome Data:

[Dementia Questionnaire/clinical mental status exam and clinical evaluation]

500 individuals were identified with incident dementia with 344 having a primary or secondary diagnosis of AD. A total of 108 individuals who reported exposure to pesticides were later diagnosed with dementia. The severity of dementia

A significantly increased risk of dementia or AD was observed in subjects exposed to any pesticide (HR=1.38 and 1.42 respectively). However, with regards to specific pesticides the only significant increased risk was observed for AD and exposure to OP pesticides (HR=1.53; p=0.03). The authors suggested that the effect of pesticide exposure may be more specific to AD and not other forms of dementia.

Cox proportional hazard ratios (HR) for dementia or AD (italics) incidence by pesticide exposure
<table>
<tr><th rowspan="2">Model</th><th rowspan="2">No dementia</th><th rowspan="2">Incident dementia/ AD</th><th colspan="2">Unadjusted models</th><th colspan="2">Adjusted models[a]</th></tr>
<tr><th>HR (95% CI)</th><th>p Value</th><th>HR (95% CI)</th><th>p Value</th></tr>
<tr><td>Any pesticide exposure[b]</td><td>2582
2738</td><td>500
344</td><td>1.25(1.01-1.55)
1.15(0.88-1.49)</td><td>0.04
0.32</td><td>1.38(1.09-1.76)
1.42(1.06-1.91)</td><td>0.008
0.02</td></tr>
<tr><td>Organophosphates</td><td>2380
2513</td><td>446
313</td><td>1.17(0.88-1.55)
1.19(0.85-1.67)</td><td>0.29
0.32</td><td>1.31(0.96-1.78)
1.53(1.05-2.23)</td><td>0.09
0.03</td></tr>
<tr><td>Organochlorines</td><td>2327
2460</td><td>439
306</td><td>1.23(0.91-1.67)
1.18(0.81-1.71)</td><td>0.18
0.39</td><td>1.33(0.96-1.85)
1.49(0.99-2.24)</td><td>0.09
0.06</td></tr>
</table>
[a]Models are adjusted for baseline age centred at 65, sex, education level (years), baseline Modified Mini-Mental State Examination score, and APOE ϵ4 status (1 or more APOE ϵ4 alleles vs. none)</td><td>Strengths
Large sample size.
Long follow-up period.
High response rate (90% participation at baseline).
Used a well-established method for identification and classification of dementia status.
Adequate control of confounding factors.
Investigated possible effect of multiple pesticide exposure

Limitations
Failed to evaluate characteristics of subjects who refused to participate.
Exposure assessment based on self-reports.
Possible misclassification of exposure bias due to lack of data on pesticide exposure duration and frequency of use
Findings may not be generalizable to other populations due to the religious makeup of the sample population influencing their lifestyle and general health characteristics</td><td>This study showed that US farmworking residents of an agricultural community exposed to OP pesticides had a 1.5-fold increased risk of developing AD compared to unexposed residents. However, this finding should be considered with caution due to limitations associated with the study's use of a crude exposure assessment and the likelihood of possible sampling bias due to subjects religious affiliations impacting on their lifestyle and subsequent rates of chronic disease</td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
		[b]Includes exposures that either did not fit into one of the 4 major pesticide classes or the subject's report was too nonspecific NB. Not enough data on other pesticides (carbamates and methyl bromides) were provided to analyse the specific effect of these pesticides The authors also investigated whether the risk of AD/ dementia increased in subjects reporting exposure to both OPs and organochlorines (n=177) and observed no significant findings (data not shown).		

Table 8. EXPOSURE SCENARIO AND ALZHEIMER'S DISEASE AND DEMENTIA

B. CHRONIC LOW-LEVEL EXPOSURE

Table 8.B.1. Cohort Studies

Test Name	First Author and Date
	Hayden 2010
Dementia Questionnaire	+
Modified Mini-Mental Status Examination (3MS)	+
DSM-III-R diagnostic criteria	+

Table 9:. OVERVIEW OF TESTS FOR ALZHEIMER'S DISEASE AND DEMENTIA

Clinical Neurological Evaluation

1. A neurological evaluation is a complete clinical assessment of the nervous system, which seeks to detect possible abnormalities by determining the function of relevant components of the nervous system. Alzheimer's Disease (AD), and dementia are detected clinically by a limited range of standard and well-validated tests as shown below.

Subject's history

2. Prior to any tests being conducted, a subjective assessment of possible health effects is conducted by taking the subject's history. This is necessary to help determine the type of neurological disorder and possible causes. Subject history involves collecting data on symptoms (to identify patterns and its development and progression over time), family history of AD/dementia, and any existing medical conditions, viral diseases or medications being taken. There are many symptoms that could suggest AD that varies depending on the stage or severity of the disease, which can be non-specific (see table below). These symptoms are similar to other dementias (i.e. vascular dementia, lewy body dementia, frontotemporal dementias, and HIV-associated dementia) and include behavioural disorders but chiefly cognitive deficits in which the first sign is often loss of short-term memory/ forgetfulness. These symptoms must be distinguished from those that arise in Mild Cognitive Impairment (MCI), defined as the intermediate stage between normal forgetfulness due to aging and development of AD. This is because not everyone with MCI goes on to develop AD. MCI symptoms include mild problems with thinking/memory that does not affect their everyday activities and having an awareness of being forgetful.

Common signs and symptoms of AD

Stage	**Symptoms/Signs**
Early	Difficulty performing tasks that take some thought, but used to come easily e.g. playing bridge, learning new info/routines[a]
	Getting lost on familiar routes [b]
	Language problems e.g. trouble finding names of familiar objects
	Flat mood/ losing interest in things previously enjoyed
	Misplacing items
	Personality changes/ loss of social skills
Intermediate (Generally more obvious and affect ability to take care of oneself)	Sleep pattern changes (often waking up at night)
	Delusions, depression, agitation
	Difficulty doing basic tasks e.g. preparing meals, choosing clothing, driving
	Difficulty reading, writing

Stage	Symptoms/Signs
	Forgetting details about current events
	Forgetting events in one's life history
	Losing awareness of oneself
	Hallucinations, arguments, striking out, violent behaviour
	Poor judgment and loss of ability to recognise danger
	Using wrong words, mispronunciation, confused sentences
	Withdrawal from social contact
	Difficulty reading, writing
	Forgetting details about current events
Severe	Inability to understand language
	Recognise family members
	Perform basic activities of daily living e.g eating, dressing, bathing
	Other symptoms include incontinence, swallowing problems, disability, infections, organ failure

[a] MCI subject finds it difficult to multitask or solve problems and takes longer to perform more difficult tasks
[b] MCI subject forgets recent events or conversations

Clinical examination

3. The next stage of the evaluation involves conducting a clinical (physical) neurological examination followed by more specific diagnostic tests. However, it should be noted that clinical tests only provide 85% accuracy for diagnosing AD from other forms of dementia.

4. The physician performs a complete physical assessment that includes neurological examination and the mental status exam (Table 9, Annex 4) e.g. Modified Mini-Mental Status Examination (3MS) (Teng & Chui, 1987). If the subject displays signs/symptoms suggestive of AD (listed above) more specific tests can be conducted that can help rule out other potential causes of dementia (e.g. CT or MRI scan). NB. Blood tests can also help rule out/identify potential causes of dementia. These include measuring levels of thyroid stimulating hormone, and vitamin B12. However, diagnosis is usually by performed by assigning standard diagnostic criteria.

Clinical diagnostic criteria

5. It is difficult to distinguish AD from other forms of dementia. However, the following clinical diagnostic criteria are traditionally used:

- Dementia established clinically and documented by a formal mental status examination

- Deficits in ≥ 2 areas of cognition
- Gradual onset and progressive worsening of memory and other cognitive functions
- No disturbance of consciousness
- Onset after age 40, most often after age 65
- No systemic brain disorders that could account for the progressive deficits in memory and cognition

6. NB. Deviations from this criteria do not exclude a diagnosis of AD, particularly because patients may have mixed dementia

Definitive Diagnosis

7. This is achieved posthumously at autopsy. The following pathological changes in brain tissue are commonly detected:

Change	Description
Neurofibrillary tangles	Twisted fragments of protein within nerve cells that clog up the cell and cause cell death
Neuritic plaques	Abnormal clusters of dead and dying nerve cells, other brain cells and protein
Senile plaques	Areas where products of dying nerve cells have accumulated around protein

Bibliography

URLs

1. MedlinePlus: www.nlm.nih.gov/medlineplus/ency/article/0000760.htm
2. Merck Manual Professional: www.merckmanuals.com/professional

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF CHRONIC FATIGUE SYNDROME

OVERVIEW OF 1999 COT REPORT

1. The COT evaluation of epidemiological studies published in 1999 can be found in Chapter 7 of the report (COT 1999).

2. The COT 1999 report did not specifically consider Chronic Fatigue Syndrome (CFS). However, the COT noted in the executive summary of the report that further research was necessary to establish whether the risk of more severe neurological or neuropsychiatric diseases is increased by low-level exposure to OPs.

OVERVIEW OF LITERATURE PUBLISHED AFTER 1999 REPORT

Definition of Chronic Fatigue Syndrome (CFS)

3. The UK Department of Health Working Group on CFS/ME use the term Chronic Fatigue Syndrome (CFS) along with Myalgic Encephalomyelitis (ME) in composite and note that the term CFS is widely used among clinicians while the term ME is commonly used by patients and the CFS community (CFS/ME WG, 2002). For the purpose of this review the term CFS will be used. The Working Group report that multiple definitions exist for CFS, which is considered to be a disorder that causes extreme disabling fatigue i.e. severe continued tiredness without demonstrable muscle weakness that is not relieved by rest and not directly caused by other medical conditions or underlying causes.

4. The symptoms of CFS are similar to those of the flu and other common viral infections. However, the key distinguishing factor is the duration, which typically lasts for six months or more. CFS symptoms are often reported to arise following an initial viral-like illness, the main symptom being a 'new fatigue' that prevents the sufferer from participating in ordinary daily activities. Other symptoms include muscle and joint pain/weakness, memory problems/confusion, headaches, sleep problems (as well as a sore throat, tender lymph nodes, and a mild fever (101^{o}F or more). Sufferers tend to either recover completely or never fully feel how they did before developing CFS to varying degrees.

5. Possible risk factors for CFS include, age and gender (women aged between 30 to 50 years commonly affected), having a previous illness, stress, and environmental exposures, (of which ciguatera fish poisoning and OP

exposures have been postulated as precipitating factors) (Devanur & Kerr, 2006).

Acute OP Exposure and CFS

6. For the purpose of this review, acute exposure is defined as a single incident where the exposure is continuous and may last up to a number of days, e.g. four days.

7. No study provided data on the chronic effects of acute exposure to OPs in relation to the potential development of CFS.

Chronic OP Exposure and CFS

8. For the purpose of this review, chronic exposure is defined as a multiple repeat incident with a variable length of exposure (e.g. lasting for one season up to a lifetime).

9. One study evaluated the chronic effects of prolonged low-level exposure to OPs in relation to the potential development of CFS. This cross-sectional study examined OP exposure in UK sheep farmers who believed their health had been affected by exposure to OP pesticides (Tahmaz et al 2003).

10. Below is a summary of the key study details and findings. Further information can be found in Annex 13, which present information on the study design used (Table 6), and the results, strengths/weaknesses and conclusions (Table 7).

Cross-sectional study

11. Tahmaz and colleagues (2003) originally adopted a case-control design but decided to abandon this approach after finding that CFS prevalence was similar in subjects originally identified as cases and controls. Instead, the authors conducted an exposure-response analysis by evaluating the association between measures of fatigue and exposure to OP pesticides directly (Tahmaz 2003, Table 6.B.3, Annex 13). A self-administered and previously validated questionnaire was used to collect information about CF symptoms (not CFS) in 63 sheep farmers (from an original sample of 206) who submitted reports to the Veterinary Medicines Directorate (VMD) Suspected Adverse Reaction Surveillance Scheme (SARSS) between 1985 and 2001. The questionnaire was also used to collect CF symptom data arising at the time the survey was conducted (in 2001). The reduced sample size (representing a 37% response rate) was largely due to subjects being either non-responsive, uncontactable, refusing to participate, living elsewhere, deceased or excluded for providing incomplete information. Due to confidentiality reasons, the authors were unable to further investigate reasons for non-participation among non-responders. The authors originally defined ‘cases’ as subjects who submitted reports of three of more CFS-type

symptoms and this was apparent in 26 of the 63 valid responses to the questionnaire. 'Controls' (who were matched to cases) were defined as subjects who submitted one CFS-type symptom or less and this was apparent in the remaining 37 of the 63 questionnaire responses. CF scores were calculated from responses to questions relating to fatigue, poor concentration/motivation, inactivity and flu-like symptoms, with CF scores greater than 76 being considered high and the remainder (<76) as a low score. No further information was provided re: the scoring method. A questionnaire was also used to collect exposure history information from which the authors constructed a non-validated exposure metric to estimate cumulative dermal exposure to OP pesticides (i.e. for exposures up until subjects first reported to the VMD, and up until when the survey was conducted (lifetime). The authors noted that the exposure metric (E_{sk}) expressed in units of m^2 days was considered to be directly related to the mass of the chemical passing through the skin, and was obtained by multiplying the following terms together (E_{sk}) = (C_{sk}) x (t_{sk}) x (S_{sk}): whereby (C_{sk}) denotes the relative concentration of the chemical on contaminated skin, (t) denotes the duration of exposure in days, and (S_{sk}) the area of skin contaminated, in m^2. Various assumptions were also made to address pesticide handling issues and use of protective clothing. The authors do not appear to have accounted for possible confounding factors but noted that the survey date coincided with the foot and mouth disease outbreak, which they suggested may have affected the response rates and biased the CF scores.

12. A significant association was observed between subjects lifetime exposures (geometric mean) and their CF scores (Tahmaz 2003, Table 7.B.3, Annex 13): subjects with high CF scores (n=54) tended to have high cumulative lifetime exposures (730 m^2 days[95% CI 490-1000]) compared to subjects with low CF scores (n=9) that tended to have low lifetime exposures (140 m^2 days[95%CI 30-670]) (p=0.004). Further graphical analysis of the relationship between estimated lifetime cumulative exposure and current chronic CF score provided further support subjects with high cumulative OP exposure having high CF scores.

13. The reliability of the positive findings reported in this study is limited by the fact that the study did not adequately address possible selection or recall bias, and relied solely on a non-validated exposure metric to evaluate cumulative dermal exposure to OPs. The study also failed to address possible confounding exposures to other pesticides or neuroactive agents. Furthermore, the lack of clinical verification of the self-reported CF symptoms (i.e. by ruling out other possible causes of symptoms as recommended by a widely used diagnostic clinical criteria: CDC-1994 case definition for CFS) suggests that these findings should be interpreted with caution. Members are subsequently alerted to the findings of a study by Kennedy et al (2004) which found that individuals from registers of patients held by the Organophosphate Information Network (OPIN) (Scotland), and the Pesticide Exposure Group of Sufferers (PEGS) with CFS-like symptoms who reported definite exposure to OPs, exhibited symptoms that conform to the CDC-1994 definition of CFS.

Summary

14. It is not possible to make any firm conclusions on the evidence reported from just one cross-sectional study (Tahmaz et al 2003). Furthermore, despite the study's use of a previously validated questionnaire to assess CFS-type symptoms it should be noted that there is currently no universally agreed diagnostic criteria for CFS, which relies on clinical criteria due to the absence of any definitive diagnostic tests.

15. The findings of the above study provide weak evidence for a possible association between CF and exposure to OP sheep dip. The drawbacks of the study suggest that stronger evidence, and more studies would be required to sufficiently conclude on the validity of these findings. This updates and accords with the COT 1999 conclusions with regard to the need for more suitably designed studies evaluating the risk of developing severe neurological diseases from prolonged low-level OP exposure.

Table 6. CHRONIC FATIGUE SYNDROME STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
B. Chronic Low-Level exposure									
6.B.3. Cross-sectional study									
Tahmaz	2003	UK	The source population comprised of 206 mostly male and female sheep farmers who submitted reports to the Veterinary Medicines Directorate (VMD) Suspected Adverse Reaction Surveillance Scheme (SARSS) between 1985 and 2001. The sample was further reduced to 178 subjects (due to 28 being uncontactable). However, only 66 subjects filled in the questionnaire (37% response rate), although 3 subjects were excluded on the basis of the questionnaires being incomplete. Other farmers refused to participate (n=16); or were living elsewhere or were deceased (n=7 and 5 respectively). The authors were unable to identify and evaluate reasons for non-participation due to confidentiality reasons. The authors originally adopted a case-control design and identified 81 subjects as cases if they	The authors originally identified 125 subjects as controls if they submitted reports of one CFS-type symptom or less. These subjects were matched to cases by age, gender, date of reporting and geographic location. The final number of controls used was 37	A self-administered questionnaire based on the 'Multidimensional Checklist Individual Strength (CIS) Questionnaire for Measuring Chronic Fatigue'. It comprised 41 questions that collected information about symptoms related to CF, both when the farmers originally reported to the VMD (past) and at the time of the survey (2001) (present). Questions relating to the following symptoms were queried: 'subjective feeling of fatigue'; 'low concentration'; 'poor motivation'; 'lack of physical activity'; 'having a flu in the past month'. A CF score was calculated from the responses, with scores > 76 being considered high and the remainder as having a low CF score. The authors did not elucidate further on the scoring method but note that the questionnaire provided an assessment of CF and not CFS. The authors considered the	Chronic fatigue	A self-administered questionnaire (based on an Institute of Occupational Medicine questionnaire) was used to collect information on subjects history of repeated OP exposure. Cumulative dermal exposure to OP pesticides (m^2 days) was estimated via the use of an non-validated exposure metric (E_{sk}) that considered the following variables: (i) duration of exposure (t) in days (i.e. from first contact with sheep dip until: (a) 2001 when the survey was carried out; or (b) from the time when the subject first reported to the VMD – an overall CF score was calculated for both time points); (ii) area of skin contaminated (m^2) (S_{sk}); and (iii) the concentration of chemical on contaminated skin (C_{sk}) (based on relative measures in which the	The authors reported that the foot and mouth disease outbreak coincided with the period when the questionnaires were sent out and suggest that this may have affected the response rate and biased the CF scores	The authors measured change in CF score with either lifetime cumulative exposure or exposure since the original report was sent to the VMD. Geometric mean (GM) exposure for the high and low CF score categories and their 95% CIs were calculated; p values reported. Tertiles of cumulative lifetime exposure were constructed (not clearly reported) in which the bottom tertile = <400 m^2 days)

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			submitted reports of three or more CFS-type symptoms. However, the study design was later abandoned due to the prevalence of CFS being similar between controls. It was decided that a cross-sectional design be used to analyse the association between measures of fatigue and exposure to OP pesticides directly. Of the 63 valid responses 26 were original designated cases.		questionnaire was a more appropriate tool to assess CF than the VMD SARRS database as it was originally developed for this purpose		dilute dip was assigned a concentration of unity and concentrated pesticide was assigned a concentration of 500). The authors noted that the exposure metric expressed was considered to be directly related to the mass of the chemical passing through the skin, and was obtained by multiplying the following terms together (E_{sk}) = (C_{sk}) x (t_{sk}) x (S_{sk}).The authors also incorporated factors associated with the subject's job title and work activities into the exposure metric (i.e., use of personal protective clothing, inhalation during dipping, post dip handling; splashing of/exposure to concentrate and accidental emersion in dip bath). To estimate the total surface area of the skin exposed to an OP contaminant, the authors made a number of assumptions that involved estimating the body parts considered to be exposed during different activities and the efficiencies of protective clothing.		

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
							NB. The authors note that the exposure metric was based on sound scientific principles		

Table 7. CHRONIC FATIGUE SYNDROME RESULTS AND CONCLUSIONS

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td colspan="5">B. Chronic Low-Level Exposure</td></tr>
<tr><td colspan="5">7.B.3. Cross-sectional study</td></tr>
<tr><td>Tahmaz</td><td>2003</td><td>[SHEEP FARMERS EXPOSED TO OP PESTICIDES WHO SUBMITTED REPORTS TO THE VMD SURVEILLANCE SCHEME]

<u>Exposure Data:</u> The authors did not provide details of tertiles of cumulative lifetime exposure or elaborate on the type of OPs used.

<u>Health Outcome Data</u>:

[Questionnaire]

No significant difference was observed between high and low CF score groups and cumulative exposure up to the time of reporting. However, a significant difference was apparent for cumulative exposure over the subject's lifetime (P=0.004).

Geometric mean estimated exposure for high and low CF categories
<table>
<tr><th></th><th>CF score</th><th>n</th><th>GM (m^2 days)</th><th>95% CI (m^2 days)</th><th>P value</th></tr>
<tr><td>Cumulative exposure until reporting to the VMD</td><td>High</td><td>57</td><td>550</td><td>350-860</td><td></td></tr>
<tr><td></td><td>Low</td><td>6</td><td>520</td><td>200-1400</td><td>0.930</td></tr>
<tr><td>Cumulative exposure over lifetime</td><td>High</td><td>54</td><td>730</td><td>490-1000</td><td></td></tr>
<tr><td></td><td>Low</td><td>9</td><td>140</td><td>30-670</td><td>0.004</td></tr>
</table>
The authors noted that the main reason for the change in association between CF score and exposure between the original report and the time of the survey was due to significant reduction in CF score for subjects with lower lifetime exposures (i.e. bottom tertile of <400 m^2 days) P=0.005 (graphically presented).

The authors analysed the relationship between estimated cumulative exposure to OP pesticides over the subject's lifetime and CF score at present (graphically presented). Pearson correlation coefficient between CF score and cumulative exposure for whole dataset (cases and controls) was 0.24 (P=0.058). Exposure was relatively low for most people, although their accompanying CF score was mostly high (i.e. above 76 and thus considered to be a probable fatigue case). All subjects with high cumulative exposure to OPs had high CF scores. Group analysis showed that subjects originally selected as controls had slightly higher exposures on average but similar pattern of CF</td><td><u>Strengths</u>
Exposure response analysis conducted.
Assessed dermal exposure via use of a complex exposure metric that accounted for different exposure scenarios/conditions

<u>Limitations</u>
Small sample size.
Low response rate (37%).
Possible recall bias.
Inadequate account of potential confounding factors.
Possible selection bias
Exposure metric was not validated</td><td>This study abandoned its previous case-control design due to a lack of differences in CF prevalence between the study groups, and chose to directly analyse the association between measures of fatigue and exposure to pesticides. The findings appear to suggest an association exists between lifetime exposure to OPs and self-reports of symptoms consistent with CFS in people who consider their health was affected by pesticides; higher fatigue scores were associated with higher exposure to OPs. However, the study was subject to various limitations that reduce the strength of these findings</td></tr>
</table>

Table 8. EXPOSURE SCENARIO AND CHRONIC FATIGUE SYNDROME

B. CHRONIC LOW-LEVEL EXPOSURE

Table 8.B.3. Cross-sectional study

Test Name	First Author and Date
	Tahmaz 2003
Multidimensional checklist individual strength (CIS) Questionnaire for Measuring CF	+

Table 9. OVERVIEW OF TESTS FOR CHRONIC FATIGUE SYNDROME

Subject's History

1. Prior to any tests being conducted, a subjective assessment of possible health effects is conducted by taking the subject's history. This is necessary to help determine the type of condition and possible causes. Subject history involves collecting data on symptoms (to identify patterns and its development and progression over time), family history of illnesses, and any existing medical conditions, viral diseases or medications being taken. Several non-specific symptoms could suggest CFS and these resemble the flu and other common viral infections. However, the symptoms tend to arise following an initial viral-like illness (e.g. EBV or HHV-6) but last for a minimum of six months.

2. The main CFS symptom is a new fatigue that prevents the patient from taking part in ordinary daily activities.

3. Other symptoms include:

- Muscle pain
- Memory problems/confusion
- Headaches
- Pain in multiple joints
- Sleep problems
- Sore throat
- Tender lymph nodes
- Mild fever (≤ 101^{o}F)

Clinical Examination

4. There is currently no definitive diagnostic test for CFS. Therefore, diagnosis is generally made by ruling out other possible illnesses via a thorough physical and mental health examination followed by a series of laboratory screening tests to help rule out/identify other possible causes of symptoms. A reasonable laboratory assessment would include following tests:

- CBC
- Measurement of electrolytes
- ESR
- Thyroid stimulating hormone
- Chest x-ray
- Anti-nuclear antibody (ANA) test
- Rheumatoid factor test
- Hepatitis
- HIV

5. A diagnosis is made via the use of clinical criteria. Examples include the Multidimensional Checklist Individual Strength (CIS) Questionnaire for Measuring Chronic Fatigue (Beursken et al 2000), and the CDC 1994 case definition

Diagnostic Criteria for CFS

6. The following case definition used by the CDC is not universally agreed (and therefore not strict):

Unexplained, persistent, or relapsing chronic fatigue with all of the following characteristics:
• Lasts for ≥ 6 mo • Is new or has a definite onset • Is not due to ongoing exertion • Is not substantially alleviated by rest • Substantially reduces occupational, educational, social, or personal activities
At least 4 of the following for ≥ 6 mo*:
• Impaired short-term memory (self-reported) severe enough to substantially reduce occupational, educational, social, or personal activities • Sore throat • Low-grade fever • Tender, enlarged, painful cervical or axillary lymph nodes • Muscle pain • Abdominal pain • Multijoint pain without joint swelling or tenderness (arthralgia) • Headaches that are new in type, pattern, or severity • Unrefreshing sleep • Postexertional malaise lasting > 24 h • Cognitive difficulties (especially with concentrating and sleeping)

*Must not predate the fatigue.
Data from the Centers for Disease Control and Prevention, the National Institutes of Health, and the International Chronic Fatigue Study Group

7. Having depression or severe anxiety excludes the diagnosis of CFS.

Bibliography

URLs

1. MedlinePlus: www.nlm.nih.gov/medlineplus/
2. Merck Manual Professional: www.merckmanuals.com/professional
3. CDC - Chronic Fatigue Syndrome : www.cdc.gov/cfs/general/index.html

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EVALUATION OF MAGNETIC RESONANCE IMAGING ABNORMALITIES

INTRODUCTION

1. Magnetic resonance imaging (MRI) studies were not considered by the COT as part of the COT 1999 review. MRI studies are available for subjects exposed to sarin during the 1995 Tokyo subway incident and the 1991 Gulf War Khamisiyah incident. A single case-report of acute exposure to dichlorvos was also retrieved.

OVERVIEW OF LITERATURE CONSIDERED

2. MRI studies report on chronic neuroanatomical changes following acute exposure to OPs. Exposure to sarin during the Tokyo subway incident involved 5,500 victims, of which 12 died, 641 were seen at St Luke's Hospital on the day of the incident and 349 were seen the following week. On the day of the attack, 5 patients were critically poisoned, 106 were classified as moderate (requiring an overnight stay in hospital) and 531 were treated as outpatients but with acute cholinergic symptoms (Ohbu et al 1997). Acute exposure during the Gulf War Khamisiyah incident was considerably lower being classified as 'uncertain low dose or specific estimated subclinical exposure' (Gray et al 1999).

3. Three studies investigating MR imaging in Tokyo subway victims were retrieved (one case-control study, and two cross-sectional studies). Two cross-sectional studies investigated MR imaging in Khamisiyah incident victims were retrieved. A further case-report of chronic MRI changes following acute poisoning with dichlorvos was also retrieved.

4. There were no studies of chronic exposure to OPs and MRI studies retrieved.

ACUTE EXPOSURE WITH CHRONIC EFFECTS

Studies from the Tokyo Subway Incident

Case-control study

5. MRI scans were analysed from 38 Tokyo subway victims selected from a group of 191 victims who attended St Luke's hospital who had been included in a previous epidemiological study. MRI scans were performed 5-6 years after the incident (Yamasue 2007). A control group of 76 healthy volunteers taken from hospital staff and their acquaintances were matched for age, sex handedness,

socioeconomic status and parental socioeconomic status. MR images were prepared using 3D spoiled gradient recalled acquisition with steady state imaging (3D-SPGR) for voxel-based-morphometry (VBM) (Annex 14, Table 6.A.2.). The investigators reported a number of findings including reduction in regional gray matter in right insular, right temporal cortex and left hippocampus. The reduction in regional white matter in the left temporal stem. The regional volume in left subsinsular was correlated with serum cholinesterase at the time of the incident. Regional white matter of the left temporal stem was related to long lasting somatic complaints in victims. The location of neuroanatomical correlates of somatic complaints was close to the insular cortex. All subjects also underwent Magnetic Resonance Diffusion Tensor Imaging (DTI). DTI showed microstructural white matter integrity was significantly disrupted in widespread bilateral regions including parietal lobe, temporal stem and brainstem. The authors suggest regional volume reduction in insular cortex is related to sarin intoxication and chronically influences subjects feeling states such as tiredness, palpitation, and bodily pain (Annex 14, Table 7.A.2.).

6. The authors report that a diagnosis of Post Traumatic Stress Disorder (PTSD) had been made in 9 OP-exposed cases (1 current, 8 former). In a previous cross-sectional study by Yamasue et al 2003, a significant reduction in the left anterior cingulate cortex volume was reported in Tokyo subway incident victims with PTSD compared to victims without PTSD. Among patients with PTSD, there was a significant negative correlation between total score of Clinician-Administered PTSD Scale (CAPS) and gray matter density in the left anterior cingulate cortex gray matter. The available data suggests the study of Tokyo victims with PTSD published in 2003 used the same subjects that were included in the study of sarin exposure published in 2007 (Annex 14, Table 7.A.2.).

7. Thus, overall, the study by Yamasue et al 2007 reports altered regional neuroanatomical changes in OP-exposed victims of the Tokyo sarin subway incident. The study was limited by low response rate (approximately 20%) and inclusion of subjects with PTSD in the exposed study group.

Cross-sectional study

8. Araki et al 2005 published MRI imaging data from Tokyo subway victims, 5-6 years after the incident, using eight subjects with or thirteen subjects without a diagnosis of PTSD (Annex 14, Table 6.A.3.). A positive correlation between P300 amplitude (at Pz) and gray matter density in the left anterior cingulate cortex (ACC) gray matter was reported in subjects with PTSD. This result was suggested to reflect reduced ACC gray matter may underlie the auditory selective attention deficits indexed in P300 in patients with PTSD. There was no correlation between P300 amplitude and ACC gray matter in subjects without PTSD. This study used the same subjects as investigated by Yamasue et al 2003 (Annex 14, Table 7.A.3.).

Studies from the Gulf War Khamisiyah Incident

Cross-sectional studies

9. MRI scans were analysed from 26 Gulf War veterans selected from the Deven's cohort Time 3 subset of 220 Khamisiyah incident veterans (Heaton et al 2007). Based on plume modelling of exposure, thirteen of these subjects were classified as either exposed or un-exposed. Unit level cumulative exposure to sarin was reported to be low between 0.035-0.144 mg min/m^3. MRI scans were performed 8-10 years after the incident. MR images were prepared using 3D spoiled gradient recalled acquisition with steady state imaging (3D-SPGR) for voxel-based-morphometry (VBM) (Annex 14, Table 6.A.3.). There was no difference between the two groups regarding diagnosis of PTSD (Clinician Administered PTSD Scale, CAPS) or PTSD symptomatology (Mississippi PTSD Scale score), or general health symptomatology. There was a higher prevalence of major depression in the non-exposed group (30.8%) compared to none in the exposed group (0%). Volumetric measurements obtained for white, gray matter, cerebral spinal fluid, right/left ventricular volume did not differ significantly between the two groups either unadjusted or adjusted for intracranial volume. Cohen's d was relatively small (0.14-0.29). Tests for linear trend (based on the combined group of 26 veterans) revealed a significant association between estimated unit level sarin/cyclosarin exposure and reduced volumetric measurements of white matter, after controlling for age and PTSD symptomatology. The authors reported the same pattern of results if subjects reporting occupational exposure pesticides either during or after the Gulf War were excluded. Regional changes in CNS neuroanatomy were not investigated (Annex 14, Table 7.A.3.).

10. Chao et al 2010 analysed MRI scans of 37 Khamisiyah incident Gulf War veterans (drawn from a group of 230 Gulf War veterans who had participated in a study of Gulf War illness). A control group of 40 veterans was drawn from the remaining 190 veterans of similar age, sex, handedness, educational level and with similar Clinician Administered PTSD Scale (CAPS). Unit level cumulative exposure to sarin was reported to be between 0.047-0.899 mg min/m^3. MRI scans were performed 11-16 years after the incident. MR images were prepared using a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence, yielding T1- weighted images (Annex 14, Table 6.A.3.). Exposed veterans had less gray matter and hippocampal volumes. No changes in total white matter, ventricular volume or cerebrospinal fluid volume were reported. An exploratory analysis of statistical trends (at uncorrected threshold of p=0.001) revealed regions of reduced gray matter density in the frontal, parietal and occipital cortices in sarin/cyclosarin exposed veterans compared with unexposed veterans. The authors investigated the relationship between neuropsychological and structural imaging data. Sarin/cyclosarin exposed veterans had a positive correlation between hippocampal volume and performance in the verbal comprehension test. Total gray matter in exposed veterans was positively correlated with the Controlled Oral Word Test (COWAT; a test of memory) and Block Design (a test of executive function) and negatively correlated with time to complete Trails A (a test of attention). and time to complete placement of pegs in groove pegboard (a psychomotor function test) (Annex 14, Table 7.A.3.).

11. Both of these studies may have been limited by low response rate (approximately 11% in each study) bias and possible misclassification of exposure. The two studies gave contrasting results regarding reductions in white matter in the

study by Heaton et al 2007, and reductions in gray matter in the study by Chao et al 2010.

12. Chao and colleagues proposed that the findings in these two studies were complimentary and consistent with the findings of the Tokyo subway incident published by Yamasue et al 2007. It was argued, by these authors, that the neuroanatomical changes in Khamisiyah Gulf War veterans involve the boundary of gray and white matter and that the different segmentation algorithms used could result in the differences reported by Chao et al 2010, (gray matter reduction) and Heaton et al 2007 (white matter reduction). A number of factors were cited which may potentially cause tissue misclassification from gray to white matter including vascular changes, myelin changes, inflammation, iron accumulation. Further studies involving different imaging modalites were recommended, such as Diffuse Tensor Imaging (DTI) and susceptibility weighted imaging.

13. It was noted that both studies controlled for the occurrence of PTSD. Incomplete adjustment might possibly affected the results, since MRI studies of Gulf War veterans have shown PTSD in these individuals is associated with neuroanatomical changes (reduced anterior cingulate cortex) (Woodward et al 2006). Neuroanatomical changes in the brainstem and basal ganglia have also been reported in Gulf War veterans who report impaired health (syndromes described as 'impaired cognition, 'confusion-ataxia' and 'central pain' (Haley et al. 2000).

Case-report

14. A case report of a 40-year old man (who drank dichlorvos) presenting with extra pyramidal symptoms like chorea, flexion, neck dystonia, tongue tremors, dysthartia and postural instability was published. T2 weighted scans showed low signal intensity surrounding a central region of high intensity in the anteromedial globus pallidus (gliosis), producing an eye-of-tiger appearance. This typical radiological sign has been described in extrapyrimidal parkinsonian disorders (Srinivasan et al 2010) (Annex 14, Table 7.A.5.).

Conclusions on Acute Exposure with Chronic Effects

15. The available evidence comes from acute exposure to sarin in subjects poisoned during the Tokyo subway incident in 1995 and also exposure to sarin/cyclosarin in Gulf War veterans resulting from proximity to the Khamisiyah munitions explosion in March 1991. There was evidence for regional reduction in gray and white matter in one study of Tokyo subway victims, but these changes are possibly confounded by the occurrence of PTSD in some of these subjects (Yamasue et al 2003). There was also evidence for neuroanatomical changes in Gulf War veterans exposed to OPs at Khamisiyah but the results of the two available studies were inconsistent and possible confounding by the occurrence of PTSD in these subjects cannot be excluded (Heaton et al 2007, Chao et al 2010). In addition, more widespread neuroanatomical changes have been found in Gulf War veterans (Haley et al 2000).

16. A single case-report of a subject who drank dichlorvos is available which showed changes in the gliosis (Srinivasan et al 2010). There were no other data to

confirm such a change following acute exposure to OPs (Annex 14, Table 6.A.5. and Table 7.A.5.).

CHRONIC LOW-LEVEL EXPOSURE

17. No studies of MRI following chronic low-level exposure to OPs were identified.

Table 6. MAGNETIC RESONANCE IMAGING STUDY DESIGN DETAILS

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
A. Acute Exposure with Chronic Effects									
6.A.1. Cohort studies - none									
6.A.2. Case-control study									
Yamasue	2007	Japan	191 out of 582 victims who participated in a previous study were eligible. 38 agreed to participate in a neuroimaging study in 2000-2001, 5-6 years post incident. Exclusion for both cases and controls included presence or history of neurological illness, traumatic brain injury with any know cognitive consequences or loss of consciousness for 5 minutes, history of electroconvulsive therapy, substance abuse or addiction history.	76 healthy volunteers.from hospital staff and their acquaintances Additional exclusion criteria included history of psychiatric disease or family history of axis I disorder* in first degree relatives (*Clinical disorders, including major mental disorders, and learning disorders, Substance Use Disorders)	MRI scans were obtained from a 1.5 T scanner using 3D spoiled gradient recalled acquisition with steady state imaging (3D-SPGR) for voxel-based-morphometry (VBM) and used SPM2 involved spatial normalisation, extraction of gray and white matter from normalised images and analysis of regional gray and white matter volume across the whole brain. Normalised images were averaged and smoothed with an 8 mm Gaussian kernel. In a second step each image was deformed to a study-specific template. In the final step a modified mixture model cluster analysis corrected images for non uniformities in signal intensity and partitioned into gray matter, white matter and cerebrospinal fluid and background. In an intensity modulation step, voxel values of the segmented images were multiplied by the measure of warped and	MRI scans of subjects acutely poisoned with sarin during the Tokyo subway incident.	None reported, but all subjects acutely poisoned.	Cases and controls matched for age, sex handedness, socioeconomic status and parental socioeconomic status Group comparisons used intracranial volume as a confounder in addition to age and sex.	Vicitms and controls compared by one-way ANOVA. Multiple linear regression. or ANCOVA (using serum ChE levels and clinical symptoms (somatic reports, ocular problems and forgetfulness), age and gender. A further ANCOVA investigated the effects of psychological stress on gray matter density. Statistical parametric map of the t statistic [SPM (t)} were displayed (uncorrected p<0.001 for graphical reporting.

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
					un-warped structures derived from the non-linear step of normalisation. This step converted relative regional gray matter density to absolute gray matter density. The resulting images were smoothed with a 12 mm Gaussian kernel. All subjects also underwent Diffusion Tensor Imaging. A single-shot, spin echo echo-echo planar sequence. Diffusion-weighted MRI images was corrected on each T2-weighted echo-echo planar image. A fractional anisotropy (FA) map and mean diffusivity were generated. The FA template specific to this study was created from all participants. Each T2-weighted echo-echo planar image was coregistered into the 3D-SPGR image and the coregistration parameter was applied to the corresponding FA map. The normalisation parameter for £D-SPGR images was applied to the FA map. Normalised FA maps were smoothed aith an 8 mm isotropic Gaussian kernel and a mean image FA template created. All FA maps in native space were transformed onto the sterotatic space by registering images to the customised FA template.				Results for volumes that survived a correction at 0.05 reported). Tabulated data were transformed to Z scores. Small volume correction for multiple comparisons was used for the bilateral hippocampus (where previous animal studies reported changes induced by sarin intoxication) In correlational analyses, intracranial volume, age, sex as confounding variables and serum ChE on day of attack and/or severity of clinical symptoms as the covariate of interest. The investigators explored regions associated with psychological stress (PTSD and psychological

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
					Normalised FA map was smoothed with a 12 mm isotropic Gaussian kernel.				impact of the event) was explored within the cluster where group comparisons reported regional volume reductions in victims. Statistical comparison between the two groups using FA maps was tested using SPM2. Age and sex were treated as confounders. Uncorrected p<0.001, corrected p<0.05.
6.A.3. Cross-sectional studies									
Araki	2005	Japan	47 subjects (24 male, 23 female) mean age 42.1 years (SD 13.2 y). 10 with unequivocal PTSD related to Tokyo subway sarin attack, 2 with current psychiatric co morbidity (depression and panic disorder with agoraphobia) excluded. Final group of 8 (5 male, 3 female) with PTSD	From same group of 47 subjects, 19 with equivocal PTSD excluded. From remaining 18, a further five excluded (insomnia and unsuccessful ERP) Final group of 13 without PTSD (8 male , 5 female). All subjects had been treated for acute sarin intoxication	MRI data were obtained from 6/8 victims with PTSD using a 1.5-T scanner. Three dimensional Fourier-transformed spoiled gradient recalled acquisition with steady state was implemented. Image analysis used ANALYZE PC 3.0 and SPM software running in MATLAB 6.1. Normalised images were segmented into gray, white matter, cerebral spinal fluid and skull/scalp compartments using an automated	MRI imaging data, P300 ERP data, clinical measures (IES-R, STAI scores) and serum cholinesterase	None reported but all subjects had acute OP intoxication.	Age, gender and scores on IES-R, STAI (measures of psychiatric status), subject and parental socio-economic status were not significantly different between groups. None had received psychiatric treatment or medication before participation or a history of	Regional voxel-based analyses of the images in SPM after co-varying for global normalisation, and these analyses can be regarded as analysis of covariance. P300 amplitudes were treated as covariates of interest. To test hypotheses about regionally

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			All subjects had been treated for acute sarin intoxication		process. The Spatially normalised segments of gray and white matter were smoothed with a 12-mm full-width at half-maximum isotropic Gaussian kernel to accommodate individual variability in the sulcal and gyral anatomy. This generated a spectrum of gray and white matter intensities which is equivalent to weight average of gray or white matter voxels located in the volume defined by the smoothing kernel and was considered to represent local amount of gray or white matter.			neurological illness, serious head trauma or any known cognitive consequences, loss of consciousness for more than five mins or alcohol or substance abuse. Serum cholinesterase measured	specific covariate effects, the estimates were compared using linear contrasts (positive or negative correlation). The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic (SPM (t)). The (SPM (t)) were transformed to the normal distribution (SPM (z)) and thresholded at P<0.001. The significance of each region was estimated by distributional approximations from the theory of random Gaussian fields. The correlational ananlysis was performed to examine the possible association between smaller anterior cingulate cortex reported in a previous study, small vo9lume correction was

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									applied using the maxima obtained in the previous study as the centre of a small volume (113 voxels
Chao	2010	USA	Participants were drawn from 230 Gulf war veterans who participated in a study of Gulf war illness between 2002-2007. 40 identified as having been potentially exposed to sarin/cyclosarin at the Khamisiyah incident according to plume modelling. MRI data not available for 3 subjects due to claustrophobia.	From remaining 190 veterans, 40 of similar age, sex, handedness, educational-level and with similar Clinical-Administered PTSD Scale (CAPS) scores with exposed veterans served as controls.	MRI data were aquired on a clinical 1.5-T MR scanner. The MRI protocol consisted of double spin-echo (DES) sequence yielding proton density and T2-weighted MR images and a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence, yielding T1-weighted images. Individual T1 images were segmented with the default unified segmentation algorithm available in SPM8. maps of gray matter, white matter, cerebral spinal fluid and intracranial volumes. Spatially normalised and modulated maps of gray and white matter, smoothed with a 10 mm Gaussian kernel were used in voxel-based morphometry analyses Hippocampal volumes were traced semi-automatically on MRPAGE images. Visual inspection and correction was undertaken. Right and left hippocampal volumes combined.	MRIs for gulf war veterans exposed to sarin/cyclosarin	No exposure data reported. Study relied on modelling of exposure to provide unit level estimates for the exposure to sarin/cyclosarin during the Khamisiyah incident in March 1991	No significant differences between exposed, and unexposed veterans in gender, age, education, handedness, PTSD symptomatology (as assessed by CAPS), diagnosis of PTSD, major depressive disorder ro chronic multi system illness. Analyses used age, sex and Intracranial volume as confounding covariates.	Demographic and descriptive characteristics were compared across the dichotomous groups using Kruskai-Wallis for continuous variables and chi-squared for cetagorical variable. Kruskai-Wallis were used to compare factor analysis-derived syndromes across the two exposure groups. Three analyses were conducted to examine the association between sarin/cyclosarin exposure and volumetric MRI measures. 1) ANCOVAused to examine group differences in volumetric measures with age, sex and

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									intracranial volume (ICV) (gray+white matter+cerebral spinal fluid+hippocampal volume) 2) partial correlations controlling for age sex and ICV used to assess the relationship between unit level dose-estimates and total gray, white matter, cerebral spinal fluid and hippocampal volumes. 3) Linear trend modles with individual unit-level dose estimates as the independent variables and adjustments for age, sex and ICV. Because the voxel-wise statistical parametirc map (SPM) comprises the result of many statistical tests, is necessary to correct for multiple dependent comparisons.

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									The Family Wise Errors method was used. Spearman's correlation coefficients were used to assess the relationship between neuropsychological test performance and total gray, white matter and hippocampal volumes.
Heaton	2007	USA	Participants were drawn from a subset of the Deven's cohort (2949 veterans). The Time 3 group (fall 1994-summer 1996) was a stratified random sample of 220 veterans. Participants were recruited between 1999-2001 from the Time 3 subset.. 31 subjects (without history of head injury with loss of consciousness, major psychiatric or neurologic disorder, past/current substance abuse or presence of metal in body).	13 subjects from the 26 subjects selected for this study were considered as non-exposed to OPs arising from the Khamisiyah incidents	Conventional MR images were acquired between 1999-2001 using 1.5 T general Electric whole body device operating a software level 4.8 ATI-weighted mid sagital localizer image to determine a plane perpendicular to the anterior commissure-posterior commissure line followed by double spin echo spin echo 3 mm axial slice of the whole brain. A three-dimensional Fourier transform spoiled gradient-recalled acquisition (3DFT-SPGR) was used to generate images with good contrast between gray matter and white matter, consisting of 124- 1.5 mm thick coroneal slices. All images were processed with semi-automated 'MRX'	MRI scan and volumetric estimation of gray, white matter, cerebral spinal fluid, right and left ventricle volumes.	Unit level cumulative estimated dosages derived from plume modelling and unit level positions during 4 day period in March 1991.	Volumetric measurements were confounded by PTSD symptomatology (which was assessed using the Mississippi Scale for Combat-Related Post Traumatic Stress Disorder. This approach was considered sufficient to assess for the influence of major depression on MRI scans.	Analyses were undertaken using SPSS Version 14 & SAS Version 8. Demographic, descriptive characteristics were compare using analyses of variance for continuous variables and either chi-squared or Fischer's exact test for categorical variables. Volumetric measurements of white matter, gray matter, cerebral spinal fluid, right lateral ventricle and left lateral ventricle

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			Five failed to complete MRI protocol. The final group consisted of 26 subjects.(15 male/11 female) mean age 41.15±9.53 years (28-63 y). From this group 13 were identified as potentially exposed to OPs arising from Khamisiyah incident		software package in 2001-2003, which produced segmentation of voxels representing cerebral spinal fluid, gray matter, white matter and connective tissue of vessels. Volumteric measurements were quantified in mm^3 and converted to percentages of intracranial volume.				were compared between exposed and non-exposed groups using ANOVAs. Analyses were adjusted for age and PTSD. Linear trend tests for exposure level response assessment adjusted for age and PTSD. Further analysis to assess for the effect of self-reported pesticide exposure during and after the Gulf War on volumetric measurements of brain tissue.
Yamasue	2003	Japan	36 subjects were recruited form Tokyo subway attack victims who were treated for acute sarin intoxication with a follow-up visit at St Lukes Hospital (Tokyo). Scans were performed 5-6 years after the incident. Assessment using Clinicians Administered	16 subjects from the 36 subjects identified who never had PTSD or history of neuropsychiatric disorders	MRI acquisition using 1.5 T scanner with 3D Fourier-transform spoiled gradient recalled acquisition with steady state for volume analysis. Image analysis performed using ANALYZE PC 3.0 and SPM 99 software in MATLAB 6.1 Images were spatially normalised and then segmented into gray matter, white matter, cerebral spinal fluid skull/scalp compartments. Spatially normalised	MRI scans and volumetric data for brain regions in Tokyo subway victims with and without PTSD.	Exposure not directly reported. Study population and controls were exposed to sarin/cyclosarin during subway incident and received hospital treatment for acute intoxication.	Age, gender. In correlational analysis (age at onset, height, body weight, SES, parental SES, duration of illness, scores of revised impact of event scale and serum cholinesterase concentration.	. Statistical comparison between the two groups By ANCOVA using age and gender as confounding variates. To test hypotheses with respect to regionally specific group effects, the estimates were compared using two linear

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
			PTSD Scale (CAPS). Subjects screened for neuropsychiatric disorders. Nine diagnosed with PTSD (1 current (male), 8 (4 male/4 female) with a history of PTSD. (3 of these subjects had a diagnosis of mental illness, major depression (1), current panic with agrophobia (1), and 1 with history of panic disorder with agrophobia. Two of these subjects had received benzodiazepines for 2-4 months, 2-4 y ago for insomnia or general anxiety) 10 with partial PTSD excluded. One with alcohol dependence excluded		segments were smoothed with a 12 mm full width half-maximum isotropic Gaussian kernel				contrasts (more or less gray or white matter in patients than controls. A statistical parametric map of the t statistic [SPM (t)} were transformed to the normal distribution [SPM (z)] and with a threshold of P<0.001. The significance of each region was estimated by distributional approximations from the theory of random Gaussian fields. Small volume correction for multiple comparisons was used for regions that had been predicted in advance (hippocampus, and anterior cingulate cortex) Correlational analysis was undertaken for PTSD severity in the PTSD subjects. Global gray (or white) matter, age, and gender were

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									treated as confounding covariates and total CAPS as covariates of interest. To test hypotheses about regional specific covariate effects, the estimates were compared using two linear contrasts (Positive or negative correlation). Small volume correction was applied. Correlational analysis was also undertaken with age at onset, height, body weight, socio economic status (SES), parental SES, duration of illness, scores of revised-impact of event scale and serum cholinesterase in victims with and without PTSD separately. These clinical measures were treated as covariates of interest, and

First Author	Date	Country	Study population	Reference population	Health assessment method	Health outcome variables	Measure of Exposure	Risk factors/ bias/ confounders	Statistical analysis
									global gray (white) matter, age, gender were treated as confounding covariates
6.A.4. Case series studies - none									
6.A.5. Case report									
Srinivasan	2010	India	Case report of a 40-year old man presenting with extra pyramidal symptoms like chorea, flexion, neck dystonia, tongue tremors, dysthartia and postural instability.	None	MRI data were obtained using T2-weighted scans. Additional procedures included T2W and Fluid Attenuated inversion recovery (FLAIR) sequences.	MRI imaging data,	Subject had drank 100 ml of dichlorvos, 6 months prior to MRI assessment..	None	Method of analysis of MRI scans
B. Chronic Low-Level Exposure									
6.B.1. Cohort studies - none									
6.B.2. Case-control studies - none									
6.B.3. Cross-sectional studies - none									
6.B.4. Case series studies - none									
6.B.5. Case reports - none									

Table 7. MAGNETIC RESONANCE IMAGING RESULTS AND CONCLUSIONS

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
A. Acute Exposure with Chronic Effects				
7.A.2. Case-control studies				
Yamasue	2007	STUDY OF MRI IN TOKYO SUBWAY VICITMS (5-6 YEARS AFTER INCIDENT) EXPOSURE Not specifically reported in this study but participants had received hospital treatment for acute OP intoxication HEALTH OUTCOMES The percentage reduction of serum ChE on the day of the attack compared to the day of MRI scanning ranged from 42%-81% (62±12%) Nine out of 38 victims had diagnosed PTSD (1 current (male), 8 had history of PTSD (4 male, 4 female). 3/9 subjects with PTSD had mental co-morbidity (current major depression (1), current (1), history of (1) panic disorder with agoraphobia. One victim without PTSD ha a history of bipolar disorder. 2/9 victims with PTSD had received benzodiazepines for 2-4 months, and the victim with bipolar had received benzodiazepines and antidepressants in the past. 35 victims had never received psychiatric treatment. 29/38 victims had somatic complaints. 33/38 reported ocular problems. 21/38 reported forgetfulness (whereas memory function was preserved (scores on Weschler memory Scale-Revised ranged from 100-119)) There was no group difference in global brain volume (total gray matter, white matter, total cerebrospinal fluid or intracranial volume. Regional analyses revealed a significant regional gray matter volume reduction in the left hippocampus (also found with small volume correction). A significant regional white matter volume reduction in the left stem close to the insular cortex. The regional white matter volumes in right temporal stem and frontal lobe were also reduced (although significance was marginal (0.05< corrected p<0.1) A significant positive association with serum ChE levels at the time of incident was found in left sinsular white matter region (close to temporal stem). Serum ChE levels at time of incident also showed positive correlations with regional white matter volume in the right frontal lobe and left superior temporal regions at trend levels of significance (0.05< corrected p<0.1) There were no significant associations between regional gray matter volume and ChE indices. A significant negative correlation between reduced regional white matter volume in the left	Strengths Case-control design. Victims described as having intermediate exposure to sarin. Detailed neuroanatomical assessment using MRI and Diffusion Tensor Images (DTI). Limitations. Authors note that 9/38 cases had current (1) or past (8) PTSD which is associated with neuropathological changes. Analyses excluding these subjects were not performed.	Authors report this is the first study showing neuropathological changes in subjects with acute exposure to sarin. Reduction in regional gray matter in right insular, right temporal cortex and left hippocampus. Reduction in regional white matter in the left temporal stem. Regional volume in left subsinsular was correlated with serum cholinesterase at the time of the incident. Regional white matter of the left temporal stem was related to long lasting somatic complaints in victims. The location of

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
temporal stem and severity of somatic complaints in victims. (Severity of somatic complaints was not correlated with severity of PTSD or serum ChE levels). There were no significant correlations with severity of ocular symptoms or forgetfulness with brain structure. The F tests covering both ChE levels and somatic symptoms showed significant effects in regional volume of left subsinsular white matter ([-36,-19,4], Z=3.8 corrected p=0.004) and in the left temporal stem ([-40, -10, -10], Z =3.12, corrected p=0.04). The authors reported that the locations and statistical values of these effects are close to t tests not on other somatic symptoms, but on ChE levels.

There were no correlations between an effect of psychological stress and reduced regional brain volume

The (Fractional Anisotropy) FA map created from Diffusion Tensor Images showed reduced FA in the parietal and temporal lobes bilaterally in victims. Decreased white matter volume in the left temporal stem was reported. Regional FA in the brainstem was decreased in victims. In addition the left temporal stem and left subsinsular showed correlations with the more severe physical symptoms in victims.

<table>
<tr><th></th><th>Peak Coordinate x,y,z</th><th>Z score</th><th>Corrected p</th><th>Cluster size (mm^3) (voxel threshold p<0.001</th></tr>
<tr><td>Gray Matter Regional volume reduction</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Right insular and temporal</td><td>41, -7, -5</td><td>4.47</td><td>0.05</td><td>5,399</td></tr>
<tr><td>Left hippocampus</td><td>-21, -21, -25</td><td>4.26</td><td><0.001 (SVC)</td><td>2,845</td></tr>
</table>
No correlates with ChE level or cognitive or somatic symptoms SVC= Small volume correction

<table>
<tr><th></th><th>Peak coordinate x,y,z</th><th>Z score</th><th>Corrected p value</th><th>Cluster size (mm^3) (voxel threshold p<0.001</th></tr>
<tr><td>White matter regional</td><td></td><td></td><td></td><td></td></tr>
</table>
</td><td></td><td>
neuroanatomical correlates of somatic complaints was close to the insular cortex. DTI showed microstructural white matter integrity was significantly disrupted in widespread bilateral regions including parietal lobe, temporal stem and brainstem.

Authors suggest regional volume reduction in insular cortex is related to sarin intoxication and chronically influences subjects feeling states such as tiredness, palpitation, and bodily pain.
</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>volume reduction</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Left temporal stem</td><td>-39, -17, -9</td><td>4.76</td><td>0.01</td><td>2,582</td></tr>
<tr><td>Right temporal stem</td><td>38, -8, -12</td><td>4.41</td><td>0.09</td><td>1,239</td></tr>
<tr><td>Left frontal</td><td>-21, 49, 25</td><td>4.33</td><td>0.06</td><td>667</td></tr>
<tr><td>Correlates of serum ChE level</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Right frontal</td><td>8,40,47</td><td>4.6</td><td>0.05</td><td>382</td></tr>
<tr><td>Left superior temporal</td><td>-56,-46,5</td><td>4.53</td><td>0.07</td><td>1,081</td></tr>
<tr><td>Left subsinsular</td><td>-30, 15, 12</td><td>3.63</td><td>0.007 (SVC)</td><td>3,206</td></tr>
<tr><td>Correlates of somatic complaints</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Left temporal stem</td><td>-40, -10, -10</td><td>4.04</td><td>0.002 (SVC)</td><td>214</td></tr>
</table>
SVC= Small Volume Correction

Diffusion Tensor Analysis
<table>
<tr><td></td><td>Peak coordinate x,y,z</td><td>Z score</td><td>Corrected p value</td><td>Cluster size (mm^3) (voxel threshold p<0.001</td></tr>
<tr><td>Regional diffusion tensor fractional anisotropy (FA) reduction</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Left parietal</td><td>-20, -43, -27</td><td>7.46</td><td><0.001</td><td>59,156 (voxel threshold at corrected p,0.05)</td></tr>
<tr><td>Right temporal stem</td><td>43, -17, -9</td><td>5.3</td><td><0.05</td><td></td></tr>
<tr><td>Left temporal</td><td>-40, -40, -5</td><td>5.04</td><td><0.05</td><td></td></tr>
</table>
</td><td></td><td></td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>stem</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Brainstem</td><td>2, -27, -29</td><td>5.17</td><td>0.002</td><td>1,316</td></tr>
<tr><td>fractional anisotropy (FA) reduction Correlates with somatic complaints</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Right temporal stem</td><td>35, 0, -15</td><td>3.67</td><td>0.006 (SVC)</td><td>597</td></tr>
<tr><td>Left temporal stem</td><td>-38, 2, -16</td><td>3.33</td><td>0.02 (SVC)</td><td>83</td></tr>
<tr><td>Left subsinsular</td><td>-28, -6, 8</td><td>3.60</td><td>0.008 (SVC)</td><td>709</td></tr>
</table>
SVC= Small Volume Correction
</td><td></td><td></td></tr>
<tr><td colspan="5">7.A.3. Cross-sectional studies</td></tr>
<tr><td>Araki</td><td>2005</td><td>STUDY OF MRI IN TOKYO SUBWAY VICITMS WITH PTSD 5-6 YEARS AFTER THE INCIDENT

EXPOSURE: Not specifically reported in this study but participants had received hospital treatment for acute OP intoxication.

HEALTH OUTCOMES

Within victims with PTSD, there was a trend level positive correlation between P300 amplitude (at Pz) and gray matter density in the left anterior cingulate cortex gray matter (peak coordinate [x,y,z (mm)] = [-12,32,22], corrected P=0.077, Z score = 4.08. This result was suggested to reflect reduced ACC gray matter may underlie the auditory selective attention deficits indexed in P300 in patients with PTSD

In other regions, gray and white matter density was not significantly correlated with amplitude of P300.

There was no voxel within left ACC correlated with P300 in victims without PTSD.

There were no significant correlation between P300 at Fz and Cz and voxel densities in victims with or without PTSD</td><td>Strengths. MRI imaging in Tokyo subway victims 5-6 y post incident

Limitations. Study focused on the relevance of PTSD in Tokyo subway victims rather than exposure to OPs.</td><td>The study suggests changes in anterior cingulate gray matter and P300 amplitude in Tokyo subway victims relates to the occurrence of PTSD rather than OP exposure.</td></tr>
<tr><td>Chao</td><td>2010</td><td>STUDY OF MRI IN GULF WAR VETERANS EXPOSED TO OPs AT THE KHAMISIYAH INCIDENT 11-16 YEARS AFTER THE INCIDENT</td><td>Strengths. MRI imaging of</td><td>GW veterans had reduced total gray</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr>
<td></td>
<td></td>
<td>
EXPOSURE: Not directly assessed. Exposure based on modelling of sarin/cyclosarin dispersion following munitions explosion in relation to troop unit positions. Cumulative unit level exposure estimated to be 0.047-0.889 mg min/ m^3

HEALTH OUTCOMES

Exposed veterans had less gray matter ($F_{1,75}$=7.68, p=0.007) and hippocampal ($F_{1,74}$=6.09, p=0.016) volumes.

<table>
<tr><th></th><th>Exposed</th><th>Unexposed</th><th>F-value</th><th>p-value</th></tr>
<tr><td>Total GM</td><td>654.14 (72.92)</td><td>675.89 (67.43)</td><td>7.68</td><td>0.007</td></tr>
<tr><td>Total WM</td><td>539.28 (62.59)</td><td>521.53 (71.89)</td><td>0.70</td><td>0.95</td></tr>
<tr><td>Total CSF</td><td>386.72 (131.31)</td><td>332.58 (100.48)</td><td>2.85</td><td>0.10</td></tr>
<tr><td>Hippocampus</td><td>5.09 (0.67)</td><td>5.42 (0.69)</td><td>6.41</td><td>0.01</td></tr>
<tr><td>ICV</td><td>1571.85 (178.37)</td><td>1530.01 (175.31)</td><td>1.39</td><td>0.24</td></tr>
</table>
GM= gray matter, WM = white matter, ICV = Intracranial volume, CSF= cerebral spinal fluid.

No significant group differences in total white matter, ventricular volume or cerebrospinal fluid volume.

Partial correlations controlling for Intracranial volume (ICV), age and sex revealed no significant relationships between unit-level dose-estimates and total gray, white matter, cerebral spinal fluid or hippocampal volume. Linear trend analyses revealed no significant dose-response relationships between sarin/cyclosarin exposure and total gray, white matter, cerebral spinal fluid or hippocampal volume.

Further analysis using voxel –based morphometry (VBM) showed no significant group differences (at <0.05) after Family-Wise-Errors correction for multiple comparisons.

An exploratory analysis of statistical trends (at uncorrected threshold of p=0.001) revealed regions of reduced gray matter density in the frontal, parietal and occipital cortices in sarin/cyclosarin exposed veterans compared with unexposed veterans.
</td>
<td>Khamisiyah incident Gulf War veterans 11-16 years after the incident.

Limitations. No direct measure of exposure, with assessment based on modelled plume from explosion and unit positions during potential exposure period.</td>
<td>matter and hippocampal volumes with some evidence that measures of executive functions and visuospatial abilities were also affected.</td>
</tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>The authors investigated the relationship between neuropsychological and structural imaging data.
Spearman's correlation coefficient between measures of brain volume and neuropsychological test performance.
<table>
<tr><th></th><th>Hippocampal volume Unexposed (40)</th><th>Hippocampal volume exposed (32)</th><th>Total GM volume Unexposed (40)</th><th>Total GM volume exposed (32)</th><th>Total WM volume unexposed (40)</th><th>Total WM volume exposed (32)</th></tr>
<tr><td>WAIS –III VCI</td><td>0.28</td><td>0.48**</td><td>0.13</td><td>0.27</td><td>0.38*</td><td>0.22</td></tr>
<tr><td>TMT A</td><td>-0.18</td><td>0.12</td><td>-0.38*</td><td>-0.35*</td><td>-0.10</td><td>-0.21</td></tr>
<tr><td>COWAT, FAS</td><td>-0.17</td><td>0.18</td><td>0.02</td><td>0.61**</td><td>0.20</td><td>0.43**</td></tr>
<tr><td>WAIS III Block Design</td><td>0.14</td><td>0.29</td><td>0.36*</td><td>0.42**</td><td>-0.08</td><td>0.34*</td></tr>
<tr><td>Grooved Pegboard, non-dominant hand</td><td>-0.02</td><td>-0.25</td><td>-0.14</td><td>-0.36*</td><td>0.21</td><td>-0.12</td></tr>
</table>
GM= gray matter, WM = white matter, WAIS III VCI= Verbal comprehension test, TMT A= Trail making test A, COWAT FAS = Controlled Oral Word Association Test.

Sarin/cyclosarin exposed veterans had a positive correlation between hippocampal volume and performance in the verbal comprehension test

Total gray matter in exposed veterans was positively correlated with COWAT and Block Design and negatively correlated with time to complete Trails A. and time to complete placement of pegs in groove pegboard</td><td></td><td></td></tr>
<tr><td>Heaton</td><td>2007</td><td>STUDY OF MRI IN GULF WAR VETERANS EXPOSED TO OPs AT THE KHAMISIYAH INCIDENT 8-10 YEARS AFTER THE INCIDENT

EXPOSURE: Not directly assessed. Exposure based on modelling of sarin/cyclosarin</td><td>Strengths MRI scans of Khamisiyah incident Gulf War veterans 8-10 years</td><td>Authors report subtle but persistent CNS pathology in</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>dispersion following munitions explosion in relation to troop unit positions. Unit-level cumulative dosages ranged from 0.035 to 0.144 mg min/^{3}

All exposures were considered to be in the low category.

HEALTH OUTCOMES

There were no significant differences between exposed and non-exposed for gender, age, education handedness, or for exposure to pesticides (during and post Gulf War). There was no difference between the two groups regarding diagnosis of PTSD (Clinician Administered PTSD Scale, CAPS) or PTSD symptomatology (Mississippi PTSD Scale score), or general health symptomatology. There was a higher prevalence of major depression in the non-exposed group (30.8%) compared to none in the exposed group (0%).

Volumetric measurements obtained for white, gray matter, cerebral spinal fluid, right/left ventricular volume did not differ significantly between the two groups either unadjusted or adjusted for intracranial volume. Cohen's d was relatively small (0.14-0.29)

Tests for linear trend (based on the combined group of 26 veterans) revealed a significant association between estimated unit level sarin/cyclosarin exposure and reduced volumetric measurements of white matter, after controlling for age and PTSD symptomatology. An exposure of 0.1 mg min/m^3 was associated with a 4.6% smaller white matter volume.

After controlling for age and PTSD, an increase in 0.1 mg min/m^3 sarin/cyclosarin exposure was associated with a 0.11% enlargement of left ventricular volume and a 0.13% increase in right ventricular volume.

<table>
<tr><td></td><td>Adjusted parameter estimate*</td><td>(95% CI)</td><td>p-value</td></tr>
<tr><td>% white matter/cranial volume</td><td>-4.6</td><td>(-4.79, -4.49)</td><td><0.0001</td></tr>
<tr><td>% gray matter/cranial volume</td><td>0.83</td><td>(-1.62, 3.28)</td><td>0.5052</td></tr>
<tr><td>%CSF per cranial volume</td><td>0.22</td><td>(-0.29, 0.73)</td><td>0.3934</td></tr>
</table>
</td><td>after the incident MRI scans

Limitations. Small study of subjects with reported small exposure to sarin/cyclosarin.</td><td>veterans exposed to sarin/cyclosarin.</td></tr>
</table>

<table>
<tr><th>First Author</th><th>Date Published</th><th>Major Findings</th><th>Strengths / Limitations</th><th>Conclusions</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>% right lateral ventricle volume/cranial volume</td><td>0.11</td><td>(0.01, 0.22)</td><td>0.0288</td></tr>
<tr><td>%left lateral ventricular volume/cranial volume</td><td>0.13</td><td>(0.07, 0.19)</td><td><0.0001</td></tr>
</table>
*% change/cranial volume corresponding to 0.1 mg min/m^3

There was a non-significant trend regarding the association between loss of white matter and ventricular volume . Left (r=-.142, p 4.89) and Right (r=-3.26, p = 0.104)

Gray matter and cerebral spinal fluid not associated with sarin/cyclosarin exposure.

To assess the possible impact of self-reported pesticide exposure on volumetric measurements of brain tissue, linear trend models were re-run excluding 2 individuals with pesticide exposure during the Gulf War, and in a separate analysis excluding four individuals who reported post war occupational exposure to pesticides. The same pattern of results was produced as for the full sample.</td><td></td><td></td></tr>
<tr><td>Yamasue</td><td>2003</td><td>STUDY OF MRI IN TOKYO SUBWAY VICITMS WITH PTSD (5-6 YEARS AFTER INCIDENT)

EXPOSURE

HEALTH OUTCOMES
A significant reduction in the left anterior cingulate cortex volume was reported in victims with PTSD compared to victims without PTSD (peak coordinate [x,y,z (mm)]= (-8, 12, 32), k = 113, T score= 5.67]. There were no differences in cholinesterase between the groups.

Among patients with PTSD, there was a significant negative correlation between total score of Clinician-Administered PTSD Scale (CAPS) and gray matter density in the left anterior cingulate cortex gray matter [peak coordinate [x,y,z (mm)} = (-8, 12, 28), Z score = 4.36]

In other regions gray or white matter was not significantly correlated with total CAPS score.

In victims with and without PTSD there were no significant positive or negative correlations between regional gray- or white matter density and age at onset, SES, parental SES, scores of revised impact of event scale and concentration of serum cholinesterase.</td><td>Strengths MRI scans in Tokyo subway victims with PTSD

Limitations: Study reported on neuroanatomical effects of PTSD in OP poisoned subjects.</td><td>This small study suggests PTSD in Tokyo subway victims was associated with a reduction in anterior cingulate cortex volume.</td></tr>
</table>

First Author	Date Published	Major Findings	Strengths / Limitations	Conclusions
7.A.5. Case report				
Srinivasan	2010	T2 weighted scans showed low signal intensity surrounding a central region of high intensity in the anteromedial globus pallidus (gliosis), producing an eye-of-tiger appearance. This typical radiological sign has been described in extrapyrimidal parkinsonian disorders.	Strengths MRI scan 6 months after acute OP poisoning. Limitations. Single case-report.	Authors report this typical radiological sign has been described in extrapyrimidal parkinsonian disorders.

Table 8. EXPOSURE SCENARIO AND MAGNETIC RESONANCE IMAGING ABNORMALITIES

A. ACUTE EXPOSURE WITH CHRONIC EFFECTS

Table 8.A.2. Case-Control Studies

Test Name / Diagnosis	First Author and Date
	Yamasue 2007 Tokyo subway victims 5-6 years after incident
Global Brain volume	
Reduced Total gray matter	-
Reduced Total white matter	-
Reduced Total cerebrospinal fluid	-
Regional brain volume	
Reduced gray matter, left hippocampus	+
Reduced white matter, Insular cortex	+
Reduced white matter, frontal lobe	+
Positive association with serumChE (at time of incident)	
Left insular white matter	+
Right fronal lobe white matter	+
Left superrior temporal region, white matter	+
Negative correlation with severity of somatic complaints	
Left temporal stem	+
Fractional Anisotropy	
Reduced in pareital temporal lobes (bilateral)	+
Brain stem	+

Table 8.A.3. Cross-Sectional Studies

Test Name / Diagnosis	First Author and Date			
	Yamasue 2003 Tokyo victims with PTSD (5-6 years after incident)	Araki 2005 Tokyo victims with PTSD (5-6 years after incident)	Heaton 2007 Gulf war veterans, Khamisiyah incident (8-10 years after incident)	Chao 2010 Gulf war veterans Khamisiyah incident (11-16 years after incident)
Global Brain volume				
Total gray matter				+
Total white matter			+	
Total cerebrospinal fluid				
Increased left/right ventricular volume			+	

Regional brain volume				
Reduced gray matter, Anterior Cingulate Cortex (ACC)	+	+		
Regional white/gray matter other than ACC	-	-		
Hippocampus				+
Reduced gray matter, frontal cortex				+
Reduced gray matter, parietal cortex				+
Reduced gray matter occipital cortex				+
Correlation hippocampal volume				
Positive for verbal comprehension				+
Total gray matter correlated with				
Controlled Oral Word Test				+
Trails A				+
Total gray matter negatively correlated with				
Groove pegboard				+

Table 9. MRI SCANNING TERMS

1. MRI scanning is a very technically demanding subject. Members can obtain an overview of the technique by accessing the following internet sites:

http://www.simplyphysics.com/MRIntro.html
http://www.bbc.co.uk/learningzone/clips/radio-waves-and-mri-scans-the-electromagnetic-spectrum/1457.html
http://www.esmrmb.org/html/img/pool/03_Tintera.pdf (an overview of MRI methods used in clinical applications)
http://www.revisemri.com/ (a question/answer revision site for MRI)

2. Some of the terms in the summarised entries for the studies reviewed in Annex 9 and 10 are explained below.

DIFFUSION TENSOR IMAGING (DTI)

3. The success of diffusion magnetic resonance imaging (MRI) is deeply rooted in the powerful concept that during their random, diffusion-driven displacements molecules probe tissue structure at a microscopic scale well beyond the usual image resolution. As diffusion is truly a three dimensional process, molecular mobility in tissues may be anisotropic, as in brain white matter. With DTI, diffusion anisotropy effects can be fully extracted, characterized, and exploited, providing even more exquisite details on tissue microstructure. The most advanced application is certainly that of fiber tracking in the brain, which, in combination with functional MRI, might open a window on the important issue of connectivity. DTI has also been used to demonstrate subtle abnormalities in a variety of diseases (including stroke, multiple sclerosis, dyslexia, and schizophrenia) and is currently becoming part of many routine clinical protocols (LeBihan D et al J Of Magnetic Resonance Imaging, 13, 534-546, 2001).

FRACTIONAL ANISOTROPY (FA)

4. This is a scalar value between zero and one that describes the degree of anisotropy of a diffusion process. A value of zero means that diffusion is isotropic, i.e. it is unrestricted (or equally restricted) in all directions. A value of one means that diffusion occurs only along one axis and is fully restricted along all other directions. FA is a measure often used in diffusion imaging where it is thought to reflect fiber density, axonal diameter, and myelination in white matter. The FA is an extension of the concept of eccentricity of conic sections in 3 dimensions, normalized to the unit range.

MAGNETIZATION PREPARED RAPID ACQUISITION GRADIENT ECHO (MPRAGE)

5. (MP-GRE / MPRAGE / MP-RAGE) A fast 3D gradient echo pulse sequence using a magnetization preparation pulse like TurboFlash. Only one segment or partition of a 3D data record is obtained per inversion preparation pulse. After the acquisition, for all rows a delay time (TD) is used to prevent saturation effects. MPRAGE is designed for rapid acquisition with T1 weighted dominance. Fast gradient echoes are characterized by their rapid sampling time, high signal intensity and image contrast while approaching steady state (the echo is collected during the time when tissues are experiencingT1 relaxation). The rapid speed of the acquisition makes it an excellent alternative to breath-hold abdominal imaging, neuro, dynamic bolus, MR angiography and cardiac imaging.

MRI AND TISSUE SEGMENTATION.

6. Magnetic Resonance Imaging uses magnetization and radio waves, rather than x-rays to make very detailed, cross-sectional pictures of the brain. It has many advantages over conventional imaging techniques. Few of them are:

- High spatial resolution
- Excellent discrimination of soft tissues
- Rich information about anatomical structure

7. Just as we have for normal images, many image processing operations are defined on MR images. Of these operations, segmentation of brain from three-dimensional (3D) magnetic resonance (MR) head images has many important research and clinical applications. The segmented brain is used to visualize and quantitatively analyze anatomical and functional cortical structures. Brain-related diseases such as epilepsy and stroke are treated using Magnetic resonance imaging (MRI)-guided neural surgery. Segmented brain provide an anatomical framework for functional visualization, which finds a potential use in neuroscience research and neurosurgical planning owing to advances in function MRI (fMRI). Moreover, the use of segmented brain images is widely used in cortical surface mapping, volume measurement, tissue classification and differentiation, functional and morphological

adaptation assessment, and characterization of neurological disorders such as multiple sclerosis, stroke, and Alzheimer's disease. MRI brain segmentation has played a critical role in these technical advancements. It is also a necessary preliminary step for many other image-processing techniques, such as brain registration and warping [1].

SMOOTHING AND GAUSSIAN KERNEL

8. Smoothing typically involves convolving the functional images with a Gaussian kernel, often described by the full width of the kernel at half its maximum height (FWHM). Common values for kernel widths vary between 4 and 12 mm FWHM. Gaussian smoothing is implemented in major software packages such as SPM (Statistical Parametric Mapping, Wellcome Institute of Cognitive Neurology, University College London), AFNI (Analysis of Functional Imaging Data), and FSL (FMRIB software library, Oxford).

SPIN ECHO, DOUBLE SPIN ECHO (SE)

9. The most common pulse sequence used in MR imaging is based of the detection of a spin or Hahn echo. It uses 90° radio frequency pulses to excite the magnetization and one or more 180° pulses to refocus the spins to generate signal echoes named spin echoes (SE). In the pulse sequence timing diagram, the simplest form of a spin echo sequence is illustrated.
The 90° excitation pulse rotates the longitudinal magnetization (Mz) into the xy-plane and the dephasing of the transverse magnetization (Mxy) starts. The following application of a 180° refocusing pulse (rotates the magnetization in the x-plane) generates signal echoes. The purpose of the 180° pulse is to rephase the spins, causing them to regain coherence and thereby to recover transverse magnetization, producing a spin echo.

10. The recovery of the z-magnetization occurs with the T1 relaxation time and typically at a much slower rate than the T2-decay, because in general T1 is greater than T2 for living tissues and is in the range of 100–2000 ms.
The SE pulse sequence was devised in the early days of NMR days by Carr and Purcell and exists now in many forms: the multi echo pulse sequence using single or multislice acquisition, the fast spin echo (FSE/TSE) pulse sequence, echo planar imaging (EPI) pulse sequence and the gradient and spin echo (GRASE) pulse sequence;; all are basically spin echo sequences.
In the simplest form of SE imaging, the pulse sequence has to be repeated as many times as the image has lines. http://www.mr-tip.com/serv1.php?type=db1&dbs=Spin%20Echo%20Sequence

SPOILED GRADIENT RECALLED ACQUISITION (SPGR)

11. The SPGR pulse sequence is similar to the spoiled GRASS sequence. The spoiled gradient recalled (SPGR) acquisition in steady state uses semi-random changes in the phase of the radio frequency (RF) pulses to produce a spatially

independent phase shift. http://www.mr-tip.com/serv1.php?type=db1&dbs=spoiled%20gradient

T1 AND T2 IMAGES

12. T1 is the longitudinal relaxation time. It indicates the time required for a substance to become magnetized after first being placed in a magnetic field or, alternatively, the time required to regain longitudinal magnetization following an RF pulse. T1 is determined by thermal interactions between the resonating protons and other protons and other magnetic nuclei in the magnetic environment or "lattice". These interactions allow the energy absorbed by the protons during resonance to be dispersed to other nuclei in the lattice.

13. All molecules have natural motions due to vibration, rotation, and translation. Smaller molecules like water generally move more rapidly, thus they have higher natural frequencies. Larger molecules like proteins move more slowly. When water is held in hydration layers around a protein by hydrophilic side groups, its rapid motion slows considerably.

14. T2 is the "transverse" relaxation time. It is a measure of how long transverse magnetization would last in a perfectly uniform external magnetic field. Alternatively, it is a measure of how long the resonating protons remain coherent or precess (rotate) "in phase" following a 90° RF pulse. T2 decay is due to magnetic interactions that occur between spinning protons. Unlike T1 interactions, T2 interactions do not involve a transfer of energy but only a change in phase, which leads to a loss of coherence.

15. T2 relaxation depends on the presence of static internal fields in the substance. These are generally due to protons on larger molecules. These stationary or slowly fluctuating magnetic fields create local regions of increased or decreased magnetic fields, depending on whether the protons align with or against the main magnetic field. Local field non-uniformities cause the protons to precess (rotate) at slightly different frequencies. Thus following the 90° pulse, the protons lose coherence and transverse magnetization is lost. This results in both T2* and T2 relaxation.
http://answers.yahoo.com/question/index?qid=20080331112427AApBwwY

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

LITERATURE CONSIDERED AS PART OF THE SYSTEMATIC REVIEW

1. Abdel Rasoul, G.M., Abou Salem, M.E., Mechael, A.A., Hendy, O.M., Rohlman, D.S. and Ismail, A.A. (2008) Effects of occupational pesticide exposure on children applying pesticides. *Neurotoxicology* **29**, 833-8.

2. Adler, C.H. (1999) Differential diagnosis of Parkinson's disease. *Med Clin North Am* **83**, 349-67.

3. Al-Shehab, A., Jaghabir, A., Hamrsheh, F., Wazwaz, A. and Abdulmajid, A. (2000) Peripheral neuropathies complicating chronic low level exposure to organophosphate, carbamates and pyrethroid pesticides. *J Dirasat Med Biol Sci* **27**, 75-89.

4. Alarcon, R., Requena, M., Hernandez, A.F., Serrano, M.D.C. and Parron, T. (2011) Characterisation of poly neuropathologies in Almeria providence in regions with differing pesticide use. *Toxicol Lett* **205**, S226

5. Albers, J.W., Garabrant, D.H., Schweitzer, S.J., Garrison, R.P., Richardson, R.J. and Berent, S. (2004a) The effects of occupational exposure to chlorpyrifos on the peripheral nervous system: a prospective cohort study. *Occup Environ Med* **61**, 201-11.

6. Albers, J.W., Berent, S., Garabrant, D.H., Giordani, B., Schweitzer, S.J., Garrison, R.P. and Richardson, R.J. (2004b) The effects of occupational exposure to chlorpyrifos on the neurologic examination of central nervous system function: a prospective cohort study. *J Occup Environ Med* **46**, 367-78.

7. Albers, J.W., Garabrant, D.H., Schweitzer, S., Garrison, R.P., Richardson, R.J. and Berent, S. (2004c) Absence of sensory neuropathy among workers with occupational exposure to chlorpyrifos. *Muscle Nerve* **29**, 677-86.

8. Albers, J.W., Garabrant, D.H., Mattsson, J.L., Burns, C.J., Cohen, S.S., Sima, C., Garrison, R.P., Richardson, R.J. and Berent, S. (2007) Dose-effect analyses of occupational chlorpyrifos exposure and peripheral nerve electrophysiology. *Toxicol Sci* **97**, 196-204.

9. Ames, R.G., Steenland, K., Jenkins, B., Chrislip, D. and Russo, J. (1995) Chronic neurologic sequelae to cholinesterase inhibition among agricultural pesticide applicators. *Arch Environ Health* **50**, 440-4.

10. Amr, M.M., Halim, Z.S. and Moussa, S.S. (1997) Psychiatric disorders among Egyptian pesticide applicators and formulators. *Environ Res* **73**, 193-9.

11. Androutsopoulos, V.P., Kanavouras, K. and Tsatsakis, A.M. (2011) Role of paraoxonase 1 (PON1) in organophosphate metabolism: implications in neurodegenerative diseases. *Toxicol Appl Pharmacol* **256**, 418-24.

12. Araki, T., Kasai, K., Yamasue, H., Kato, N., Kudo, N., Ohtani, T., Nakagome, K., Kirihara, K., Yamada, H., Abe, O. and Iwanami, A. (2005) Association between lower P300 amplitude and smaller anterior cingulate cortex volume in patients with posttraumatic stress disorder: a study of victims of Tokyo subway sarin attack. *Neuroimage* **25**, 43-50.

13. Araoud, M., Neffeti, F., Douki, W., Hfaiedh, H.B., Akrout, M., Najjar, M.F. and Kenani, A. (2011) Factors influencing plasma butyrylcholinesterase activity in agricultural workers. *Ann Biol Clin (Paris)* **69**, 159-66.

14. Arima, H., Sobue, K., So, M., Morishima, T., Ando, H. and Katsuya, H. (2003) Transient and reversible parkinsonism after acute organophosphate poisoning. *J Toxicol Clin Toxicol* **41**, 67-70.

15. Aubeneau, L., Bohu, P.A., Hannequin, D. and Maltete, D. (2008) [Chronic parkinsonian syndrome after 10 years of repeated insecticide spraying]. *Rev Neurol (Paris)* **164**, 374-6.

16. Auerbach, S.M. and Spielberger, C.D. (1972) The assessment of state and trait anxiety with the Rorschach test. *J Pers Assess* **36**, 314-35.

17. Baldi, I., Filleul, L., Mohammed-Brahim, B., Fabrigoule, C., Dartigues, J.F., Schwall, S., Drevet, J.P., Salamon, R. and Brochard, P. (2001) Neuropsychologic effects of long-term exposure to pesticides: results from the French Phytoner study. *Environ Health Perspect* **109**, 839-44.

18. Barbato, A.L. (1990) Bedside evaluation of the autonomic system. In: *Clinical methods: the history, physical, and laboratory examinations. 3rd Edition*, pp. 404-406. University of Minnesota: Butterworths]

19. Bastos, A.G., de Lima, M.A. and de Oliveira, L.F. (2005) [Evaluation of patients with dizziness and normal electronystagmography using stabilometry]. *Braz J Otorhinolaryngol* **71**, 305-10.

20. Bayrami, M., Hashemi, T., Malekirad, A.A., Ashayeri, H., Faraji, F. and Abdollahi, M. (2012) Electroencephalogram, cognitive state, psychological disorders, clinical symptom, and oxidative stress in horticulture farmers exposed to organophosphate pesticides. *Toxicol Ind Health* **28**, 90-6.

21. Bazylewicz-Walczak, B., Majczakowa, W. and Szymczak, M. (1999) Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. *Neurotoxicology* **20**, 819-26.

22. Beard, J.D., Umbach, D.M., Hoppin, J.A., Richards, M., Alavanja, M.C., Blair, A., Sandler, D.P. and Kamel, F. (2011) Suicide and pesticide use among pesticide applicators and their spouses in the agricultural health study. *Environ Health Perspect* **119**, 1610-5.

23. Beckmann, R. (2000) OP sheep dips - new study sheds light on an old problem. *J Pesticides News* 3-4.

24. Beseler, C., Stallones, L., Hoppin, J.A., Alavanja, M.C., Blair, A., Keefe, T. and Kamel, F. (2006) Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. *J Occup Environ Med* **48**, 1005-13.

25. Beseler, C.L. and Stallones, L. (2008) A cohort study of pesticide poisoning and depression in Colorado farm residents. *Ann Epidemiol* **18**, 768-74.

26. Beseler, C.L., Stallones, L., Hoppin, J.A., Alavanja, M.C., Blair, A., Keefe, T. and Kamel, F. (2008) Depression and pesticide exposures among private pesticide applicators enrolled in the Agricultural Health Study. *Environ Health Perspect* **116**, 1713-9.

27. Beurskens, A.J., Bultmann, U., Kant, I., Vercoulen, J.H., Bleijenberg, G. and Swaen, G.M. (2000) Fatigue among working people: validity of a questionnaire measure. *Occup Environ Med* **57**, 353-7.

28. Bhatt, M.H., Elias, M.A. and Mankodi, A.K. (1999) Acute and reversible parkinsonism due to organophosphate pesticide intoxication: five cases. *Neurology* **52**, 1467-71.

29. Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S. and Keane, T.M. (1995) The development of a Clinician-Administered PTSD Scale. *J Trauma Stress* **8**, 75-90.

30. Brandt, T. and Strupp, M. (2005) General vestibular testing. *Clin Neurophysiol* **116**, 406-26.

31. Brillman, J. and Kahan, S. (2005) *In a page neurology.*

32. Bromberg, M.B. (2005) An approach to the evaluation of peripheral neuropathies. *Semin Neurol* **25**, 153-9.

33. Brown, T.P., Rumsby, P.C., Capleton, A.C., Rushton, L. and Levy, L.S. (2006) Pesticides and Parkinson's disease--is there a link? *Environ Health Perspect* **114**, 156-64.

34. Browne, R.O., Moyal-Segal, L.B., Zumsteg, D., David, Y., Kofman, O., Berger, A., Soreq, H. and Friedman, A. (2006) Coding region paraoxonase polymorphisms dictate accentuated neuronal reactions in chronic, sub-threshold pesticide exposure. *FASEB J* **20**, 1733-5.

35. Buchanan, D., Jamal, G.A., Pilkington, A. and Hansen, S. (2002) Clinical validation of methods of diagnosis of neuropathy in a field study of United Kingdom sheep dippers. *Occup Environ Med* **59**, 442-6.

36. Burakgazi, A.Z. and Hoke, A. (2010) Respiratory muscle weakness in peripheral neuropathies. *J Peripher Nerv Syst* **15**, 307-13.

37. Burstyn, I., Kim, H.M., Yasui, Y. and Cherry, N.M. (2009) The virtues of a deliberately mis-specified disease model in demonstrating a gene-environment interaction. *Occup Environ Med* **66**, 374-80.

38. Camarinha, C.R., Frota, S.M., Pacheco-Ferreira, H. and Lima, M.A. (2011) [Auditory temporal processing assessment in rural workers exposed to organophosphate pesticides]. *J Soc Bras Fonoaudiol* **23**, 102-6.

39. Carod-Artal, F.J. and Speck-Martins, C. (1999) [Late onset polyneuropathy due to exposure to organophosphates]. *Rev Neurol* **29**, 123-7.

40. Catano, H.C., Carranza, E., Huamani, C. and Hernandez, A.F. (2008) Plasma cholinesterase levels and health symptoms in peruvian farm workers exposed to organophosphate pesticides. *Arch Environ Contam Toxicol* **55**, 153-9.

41. CFS/ME Working Group (2002) Department of Health.

42. Chao, L.L., Rothlind, J.C., Cardenas, V.A., Meyerhoff, D.J. and Weiner, M.W. (2010) Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology* **31**, 493-501.

43. Chatterjee, M. and Sarma, P.S. (2003) Unusual neurological complications in a case of organophosphate poisoning. *Neurol India* **51**, 290-1.

44. Cherry, N., Mackness, M., Durrington, P., Povey, A., Dippnall, M., Smith, T. and Mackness, B. (2002) Paraoxonase (PON1) polymorphisms in farmers attributing ill health to sheep dip. *Lancet* **359**, 763-4.

45. Cherry, N., Mackness, M., Mackness, B., Dippnall, M. and Povey, A. (2011) 'Dippers' flu' and its relationship to PON1 polymorphisms. *Occup Environ Med* **68**, 211-7.

46. Chuang, C.C., Lin, T.S. and Tsai, M.C. (2002) Delayed neuropathy and myelopathy after organophosphate intoxication. *N Engl J Med* **347**, 1119-21.

47. Cole, D.C., Sherwood, S., Paredes, M., Sanin, L.H., Crissman, C., Espinosa, P. and Munoz, F. (2007) Reducing pesticide exposure and associated neurotoxic burden in an Ecuadorian small farm population. *Int J Occup Environ Health* **13**, 281-9.

48. Colosio, C., Tiramani, M., Brambilla, G., Colombi, A. and Moretto, A. (2009) Neurobehavioural effects of pesticides with special focus on organophosphorus compounds: which is the real size of the problem? *Neurotoxicology* **30**, 1155-61.

49. Committee on Toxicity of Chemicals in Food, C.P.a.t.E. (1999) Organophosphates. *Department of Health, London*

50. Committee on Toxicity of Chemicals in Food, C.P.a.t.E. (2007a) Minutes of meeting held 4 September 2007. *TOX/MIN/2007/05*

51. Committee on Toxicity of Chemicals in Food, C.P.a.t.E. (2007b) Letter to the COT from Advisory Committee on Pesticides requesting advice on organophosphates. *TOX/2007/29*

52. Committee on Toxicity of Chemicals in Food, C.P.a.t.E. (2009a) Minutes of meeting held 22 September 2009. *TOX/MIN/2009/05*

53. Committee on Toxicity of Chemicals in Food, C.P.a.t.E. (2009b) Minutes of meeting held 15 December 2009. *TOX/MIN/2009/07*

54. Costa, L.G., Richter, R.J., Li, W.F., Cole, T., Guizzetti, M. and Furlong, C.E. (2003) Paraoxonase (PON 1) as a biomarker of susceptibility for organophosphate toxicity. *Biomarkers* **8**, 1-12.

55. Costa, P.T.J. and McCrae, R.R. (1992) *Revised NEO personality inventory (NEO-PI-R) and NEO five-factor inventory (NEO-FFI) professional manual*, edn. Odessa, Fl: Psychological Assessment Resources Inc.

56. Cox, R.D., Kolb, J.C., Galli, R.L., Carlton, F.R. and Cook, A.M. (2005) Evaluation of potential adverse health effects resulting from chronic domestic exposure to the organophosphate insecticide methyl parathion. *Clin Toxicol (Phila)* **43**, 243-53.

57. Crawford, J.M., Hoppin, J.A., Alavanja, M.C., Blair, A., Sandler, D.P. and Kamel, F. (2008) Hearing loss among licensed pesticide applicators in the agricultural health study. *J Occup Environ Med* **50**, 817-26.

58. Dahlgren, J.G., Takhar, H.S., Ruffalo, C.A. and Zwass, M. (2004) Health effects of diazinon on a family. *J Toxicol Clin Toxicol* **42**, 579-91.

59. Das, K., Ghosh, M., Nag, C., Nandy, S.P., Banerjee, M., Datta, M., Devi, G. and Chaterjee, G. (2011) Role of familial, environmental and occupational factors in the development of Parkinson's disease. *Neurodegener Dis* **8**, 345-51.

60. Dassanayake, T., Weerasinghe, V., Dangahadeniya, U., Kularatne, K., Dawson, A., Karalliedde, L. and Senanayake, N. (2007) Cognitive processing of visual stimuli in patients with organophosphate insecticide poisoning. *Neurology* **68**, 2027-30.

61. Dassanayake, T., Weerasinghe, V., Dangahadeniya, U., Kularatne, K., Dawson, A., Karalliedde, L. and Senanayake, N. (2008) Long-term event-related potential changes following organophosphorus insecticide poisoning. *Clin Neurophysiol* **119**, 144-50.

62. Dassanayake, T., Gawarammana, I.B., Weerasinghe, V., Dissanayake, P.S., Pragaash, S., Dawson, A. and Senanayake, N. (2009) Auditory event-related potential changes in chronic occupational exposure to organophosphate pesticides. *Clin Neurophysiol* **120**, 1693-8.

63. Davies, D.R. and Ahmed, G.M.F.T. (1999) Chronic organophosphate induced neuropsychiatric disorder (COPIND): Results of two postal questionnaire surveys. *J Nutr & Environ Med* **9**, 123-134.

64. Davies, H.G., Richter, R.J., Keifer, M., Broomfield, C.A., Sowalla, J. and Furlong, C.E. (1996) The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat Genet* **14**, 334-6.

65. de Araujo, A.J., de Lima, J.S., Moreira, J.C., Jacob Sdo, C., Soares Mde, O., Monteiro, M.C., do Amaral, A.M., Kubota, A., Meyer, A., Cosenza, C.A., das Neves, C. and Markowitz, S. (2007) [Multiple exposure to pesticides and impacts on health: a cross-section study of 102 rural workers, Nova Friburgo, Rio de Janeiro State, Brazil]. *Cien Saude Colet* **12**, 115-30.

66. Delgado, E., McConnell, R., Miranda, J., Keifer, M., Lundberg, I., Partanen, T. and Wesseling, C. (2004) Central nervous system effects of acute organophosphate poisoning in a two-year follow-up. *Scand J Work Environ Health* **30**, 362-70.

67. Derogatis, L.R., Lipman, R.S. and Covi, L. (1973) SCL-90: an outpatient psychiatric rating scale--preliminary report. *Psychopharmacol Bull* **9**, 13-28.

68. Derogatis, L.R. and Melisaratos, N. (1983) The Brief Symptom Inventory: an introductory report. *Psychol Med* **13**, 595-605.

69. Devanur, L.D. and Kerr, J.R. (2006) Chronic fatigue syndrome. *J Clin Virol* **37**, 139-50.

70. Dick, F.D., De Palma, G., Ahmadi, A., Scott, N.W., Prescott, G.J., Bennett, J., Semple, S., Dick, S., Counsell, C., Mozzoni, P., Haites, N., Wettinger, S.B., Mutti, A., Otelea, M., Seaton, A., Soderkvist, P. and Felice, A. (2007a) Environmental risk factors for Parkinson's disease and parkinsonism: the Geoparkinson study. *Occup Environ Med* **64**, 666-72.

71. Dick, F.D., De Palma, G., Ahmadi, A., Osborne, A., Scott, N.W., Prescott, G.J., Bennett, J., Semple, S., Dick, S., Mozzoni, P., Haites, N., Wettinger, S.B., Mutti, A., Otelea, M., Seaton, A., Soderkvist, P. and Felice, A. (2007b) Gene-environment interactions in parkinsonism and Parkinson's disease: the Geoparkinson study. *Occup Environ Med* **64**, 673-80.

72. Dick, R.B., Steenland, K., Krieg, E.F. and Hines, C.J. (2001) Evaluation of acute sensory--motor effects and test sensitivity using termiticide workers exposed to chlorpyrifos. *Neurotoxicol Teratol* **23**, 381-93.

73. Dunn, G. (2002) Report on an Analytical study of OP sheep dip reports. *Veterinary Medicines Directorate*

74. Durrington, P.N., Mackness, B. and Mackness, M.I. (2001) Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol* **21**, 473-80.

75. Eckerman, D.A., Gimenes, L.S., de Souza, R.C., Galvao, P.R., Sarcinelli, P.N. and Chrisman, J.R. (2007) Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in Brazil. *Neurotoxicol Teratol* **29**, 164-75.

76. Elbaz, A., Clavel, J., Rathouz, P.J., Moisan, F., Galanaud, J.P., Delemotte, B., Alperovitch, A. and Tzourio, C. (2009) Professional exposure to pesticides and Parkinson disease. *Ann Neurol* **66**, 494-504.

77. Engel, L.S., Keifer, M.C., Checkoway, H., Robinson, L.R. and Vaughan, T.L. (1998) Neurophysiological function in farm workers exposed to organophosphate pesticides. *Arch Environ Health* **53**, 7-14.

78. Engel, L.S., Checkoway, H., Keifer, M.C., Seixas, N.S., Longstreth, W.T. Jr, Scott, K.C., Hudnell, K., Anger, W.K. and Camicioli, R. (2001) Parkinsonism and occupational exposure to pesticides. *Occup Environ Med* **58**, 582-9.

79. England, J.D. and Asbury, A.K. (2004) Peripheral neuropathy. *Lancet* **363**, 2151-61.

80. Farahat, T.M., Abdelrasoul, G.M., Amr, M.M., Shebl, M.M., Farahat, F.M. and Anger, W.K. (2003) Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides. *Occup Environ Med* **60**, 279-86.

81. Fiedler, N., Kipen, H., Kelly-McNeil, K. and Fenske, R. (1997) Long-term use of organophosphates and neuropsychological performance. *Am J Ind Med* **32**, 487-96.

82. Finkelstein, Y., Milatovic, D., Lazarovici, P., Ophir, A., Richter, E.D., Aschner, M., Lecht, S., Marcinkiewicz, C., Lelkes, P.I., Zaja-Milatovic, S., Gupta, R.C., Brodsky, B., Rosengarten, A., Proscura, E., Shapira, E. and Wormser, U. (2010) Peaceful use of disastrous neurotoxicants. *Neurotoxicology* **31**, 608-20.

83. Firestone, J.A., Smith-Weller, T., Franklin, G., Swanson, P., Longstreth, W.T. Jr and Checkoway, H. (2005) Pesticides and risk of Parkinson disease: a population-based case-control study. *Arch Neurol* **62**, 91-5.

84. Fletcher, T., MacLehose, R., Hurley, F., Cherrie, J., Cowie, H., Jamal, G. and Julu, P. (2005) SHAPE: Survey of health and pesticide exposure: the telephone survey.

85. Foltynie, T., Sawcer, S., Brayne, C. and Barker, R.A. (2002) The genetic basis of Parkinson's disease. *J Neurol Neurosurg Psychiatry* **73**, 363-70.

86. Fonseka, M.M., Medagoda, K., Tillakaratna, Y., Gunatilake, S.B. and de Silva, H.J. (2003) Self-limiting cerebellar ataxia following organophosphate poisoning. *Hum Exp Toxicol* **22**, 107-9.

87. Freeman, R. (2005) Autonomic peripheral neuropathy. *Lancet* **365**, 1259-70.

88. Freeman, R. (2007) Autonomic peripheral neuropathy. *Neurol Clin* **25**, 277-301.

89. Friedman, L., Barach, P., Brautbar, N. and Richter, E.D. (2007) Latency: an important consideration in gulf war syndrome. *Neurotoxicology* **28**, 1043-4; author reply 1044-5.

90. Friedman, L.S., Brautbar, N., Barach, P., Wolfe, A.H. and Richter, E.D. (2003) Creatine phosphate kinase elevations signaling muscle damage following exposures to anticholinesterases: 2 sentinel patients. *Arch Environ Health* **58**, 167-71.

91. Furlong, C.E., Li, W.F., Richter, R.J., Shih, D.M., Lusis, A.J., Alleva, E. and Costa, L.G. (2000) Genetic and temporal determinants of pesticide sensitivity: role of paraoxonase (PON1). *Neurotoxicology* **21**, 91-100.

92. Garcia-Martin, E., Martinez, C., Alonso-Navarro, H., Benito-Leon, J., Puertas, I., Rubio, L., Lopez-Alburquerque, T., Agundez, J.A. and Jimenez-Jimenez, F.J. (2010) Paraoxonase 1 (PON1) polymorphisms and risk for essential tremor. *Eur J Neurol* **17**, 879-81.

93. Garrow, A.P. and Boulton, A.J. (2006) Vibration perception threshold--a valuable assessment of neural dysfunction in people with diabetes. *Diabetes Metab Res Rev* **22**, 411-9.

94. Gatto, N.M., Cockburn, M., Bronstein, J., Manthripragada, A.D. and Ritz, B. (2009) Well-water consumption and Parkinson's disease in rural California. *Environ Health Perspect* **117**, 1912-8.

95. Gauthier, E., Fortier, I., Courchesne, F., Pepin, P., Mortimer, J. and Gauvreau, D. (2001) Environmental pesticide exposure as a risk factor for Alzheimer's disease: a case-control study. *Environ Res* **86**, 37-45.

96. Goel, D., Singhal, A., Srivastav, R.K., Verma, A. and Lamba, A. (2006) Magnetic resonance imaging changes in a case of extra-pyramidal syndrome after acute organophosphate poisoning. *Neurol India* **54**, 207-9.

97. Goldberg, D.P. and Hillier, V.F. (1979) A scaled version of the General Health Questionnaire. *Psychol Med* **9**, 139-45.

98. Goldman, S. and Tanner, C. (1998) Etiology of Parkinson's Disease. In: *Parkinson's Disease and Movement Disorders*, pp. 133-158. London: Williams and Wilkins]

99. Gomez, M.J. and Caceres, J.L. (2010) Toxicity by organophosphate insecticides of fumigators working on the dengue control campaign in Aragua state, Venezuela, 2008. *Boletín de Malariología y Salud Ambiental* **50**, 119-125.

100. Gorth-Marnet, G. (2009) Screening for neuropsychological impairment. In: *Handbook of psychological assessment. 5th Edition*, pp. 485-518. Wiley]

101. Gray, G.C., Smith, T.C., Knoke, J.D. and Heller, J.M. (1999) The postwar hospitalization experience of Gulf War Veterans possibly exposed to chemical munitions destruction at Khamisiyah, Iraq. *Am J Epidemiol* **150**, 532-40.

102. Haley, R.W., Marshall, W.W., McDonald, G.G., Daugherty, M.A., Petty, F. and Fleckenstein, J.L. (2000) Brain abnormalities in Gulf War syndrome: evaluation with 1H MR spectroscopy. *Radiology* **215**, 807-17.

103. Hancock, D.B., Martin, E.R., Mayhew, G.M., Stajich, J.M., Jewett, R., Stacy, M.A., Scott, B.L., Vance, J.M. and Scott, W.K. (2008) Pesticide exposure and risk of Parkinson's disease: a family-based case-control study. *BMC Neurol* **8**, 6

104. Hanna, P.A., Jankovic, J. and Kirkpatrick, J.B. (1999) Multiple system atrophy: the putative causative role of environmental toxins. *Arch Neurol* **56**, 90-4.

105. Hashim, H.Z., Wan Musa, W.R., Ngiu, C.S., Wan Yahya, W.N., Tan, H.J. and Ibrahim, N. (2011) Parkinsonism complicating acute organophosphate insecticide poisoning. *Ann Acad Med Singapore* **40**, 150-1.

106. Hawton, K., Simkin, S., Malmberg, A., Fagg, J. and Harriss, L. (1998) *Suicide and stress in farmers*, edn. London: TSO.

107. Hayden, K.M., Norton, M.C., Darcey, D., Ostbye, T., Zandi, P.P., Breitner, J.C. and Welsh-Bohmer, K.A. (2010) Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. *Neurology* **74**, 1524-30.

108. Heaton, K.J., Palumbo, C.L., Proctor, S.P., Killiany, R.J., Yurgelun-Todd, D.A. and White, R.F. (2007) Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology* **28**, 761-9.

109. Hernandez, A.F., Mackness, B., Rodrigo, L., Lopez, O., Pla, A., Gil, F., Durrington, P.N., Pena, G., Parron, T., Serrano, J.L. and Mackness, M.I. (2003) Paraoxonase activity and genetic polymorphisms in greenhouse workers with long term pesticide exposure. *Hum Exp Toxicol* **22**, 565-74.

110. Hogstedt, C., Andersson, K. and Hane, M. (1984) A questionnaire approach to the monitoring of early disturbances in central nervous functions. In: *The biological monitoring of exposure to industrial chemicals*, pp. 275-287. Washington: Hemisphere:]

111. Hong, S.Y., Jeong, D.S., Gil, H.W., Yang, J.O., Lee, E.Y. and Hong, S.Y. (2009) The estimation of pesticide exposure in depression scores:in case of Korean orchard farmers. *J Pest Sci* **82**, 261-265.

112. Horowitz, M., Wilner, N. and Alvarez, W. (1979) Impact of Event Scale: a measure of subjective stress. *Psychosom Med* **41**, 209-18.

113. Horowitz, S.H., Stark, A., Marshall, E. and Mauer, M.P. (1999) A multi-modality assessment of peripheral nerve function in organophosphate-pesticide applicators. *J Occup Environ Med* **41**, 405-8.

114. Hoshino, A.C., Pacheco-Ferreira, H., Taguchi, C.K., Tomita, S. and Miranda Mde, F. (2008) Ototoxicity study in workers exposed to organophosphate. *Braz J Otorhinolaryngol* **74**, 912-8.

115. Hsieh, B.H., Deng, J.F., Ger, J. and Tsai, W.J. (2001) Acetylcholinesterase inhibition and the extrapyramidal syndrome: a review of the neurotoxicity of organophosphate. *Neurotoxicology* **22**, 423-7.

116. Hughes, R.A. (2002) Peripheral neuropathy. *BMJ* **324**, 466-9.

117. Institute of Environment and Health (2005) Pesticides and Parkinson's Disease - a critical review. *IEH Web Report W21*

118. Jalali, N., Balali-Mood, M., Jalali, I. and Shakeri, M.T. (2011) Electrophysiological changes in patients with acute organophosphorous pesticide poisoning. *Basic Clin Pharmacol Toxicol* **108**, 251-5.

119. Jamal, G.A., Hansen, S., Apartopoulos, F., Peden, A., Abdul-Aziz, M. and Ballantyne, J.P. (2001) Peripheral nerve dysfunction in farmers using organophosphate sheep dip. *J Nutr Environ Med* **11**, 9-22.

120. Jamal, G.A., Hansen, S., Pilkington, A., Buchanan, D., Gillham, R.A., Abdel-Azis, M., Julu, P.O., Al-Rawas, S.F., Hurley, F. and Ballantyne, J.P. (2002a) A clinical neurological, neurophysiological, and neuropsychological study of sheep farmers and dippers exposed to organophosphate pesticides. *Occup Environ Med* **59**, 434-41.

121. Jamal, G.A., Hansen, S. and Julu, P.O. (2002b) Low level exposures to organophosphorus esters may cause neurotoxicity. *Toxicology* **181-182**, 23-33.

122. Jayasinghe, S.S. and Pathirana, K.D. (2011) Effects of deliberate ingestion of organophosphate or paraquat on brain stem auditory-evoked potentials. *J Med Toxicol* **7**, 277-80.

123. Jin, Y.H., Jeong, T.O. and Lee, J.B. (2008) Isolated bilateral vocal cord paralysis with intermediate syndrome after organophosphate poisoning. *Clin Toxicol (Phila)* **46**, 482-4.

124. Johnson, B.L., Anger, W.K., Durao, A. and Xintaras, C. (1990) *Advances in neurobehavioural toxicology: applications in environmental and occupational health*, edn. Chelsea MI: CRC Press Inc.

125. Jokanovic, M. (2001) Biotransformation of organophosphorus compounds. *Toxicology* **166**, 139-60.

126. Julu, P., Jamal, G., Fletcher, T. and Hutchinson, E. (2007) Clinical evaluation of a sample of participants in the SHAPE survey of health and pesticides exposure.

127. Kamel, F., Boyes, W.K., Gladen, B.C., Rowland, A.S., Alavanja, M.C., Blair, A. and Sandler, D.P. (2000) Retinal degeneration in licensed pesticide applicators. *Am J Ind Med* **37**, 618-28.

128. Kamel, F., Rowland, A.S., Park, L.P., Anger, W.K., Baird, D.D., Gladen, B.C., Moreno, T., Stallone, L. and Sandler, D.P. (2003) Neurobehavioral performance and work experience in Florida farmworkers. *Environ Health Perspect* **111**, 1765-72.

129. Kamel, F., Engel, L.S., Gladen, B.C., Hoppin, J.A., Alavanja, M.C. and Sandler, D.P. (2005) Neurologic symptoms in licensed private pesticide applicators in the agricultural health study. *Environ Health Perspect* **113** , 877-82.

130. Kamel, F., Tanner, C., Umbach, D., Hoppin, J., Alavanja, M., Blair, A., Comyns, K., Goldman, S., Korell, M., Langston, J., Ross, G. and Sandler, D. (2007a) Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J Epidemiol* **165**, 364-74.

131. Kamel, F., Engel, L.S., Gladen, B.C., Hoppin, J.A., Alavanja, M.C. and Sandler, D.P. (2007b) Neurologic symptoms in licensed pesticide applicators in the Agricultural Health Study. *Hum Exp Toxicol* **26**, 243-50.

132. Kara-Peketi, K., Atassime, S., Kemkoi, H., Robin, H. and Frimat, P. (2009) Chemical risk assessment and preventative procedure among cotton growers in Togo. Longitudinal survey between 2003 and 2008. *Archives Des Maladies Professionelles et de l'Environment* **70**, 634-641.

133. Kawada, T., Katsumata, M., Suzuki, H., Li, Q., Inagaki, H., Nakadai, A., Shimizu, T., Hirata, K. and Hirata, Y. (2005) Insomnia as a sequela of sarin toxicity several years after exposure in Tokyo subway trains. *Percept Mot Skills* **100**, 1121-6.

134. Kawana, N., Ishimatsu, S. and Kanda, K. (2001) Psycho-physiological effects of the terrorist sarin attack on the Tokyo subway system. *Mil Med* **166**, 23-6.

135. Keane, T.M., Caddell, J.M. and Taylor, K.L. (1988) Mississippi Scale for Combat-Related Posttraumatic Stress Disorder: three studies in reliability and validity. *J Consult Clin Psychol* **56**, 85-90.

136. Keifer, M.C., Rosenstock, L., Checkoway, H., Fenske, R. and Robinson, L. (2000) Neurological Effects of Organophosphates on Farm Workers. *Govt Reports Announcements & Index* **02**,

137. Kenemans, J.L. and Kahkonen, S. (2011) How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. *Neuropsychopharmacology* **36**, 26-51.

138. Kennedy, G., Abbot, N.C., Spence, V., Underwood, C. and Belch, J.J. (2004) The specificity of the CDC-1994 criteria for chronic fatigue syndrome: comparison of health status in three groups of patients who fulfill the criteria. *Ann Epidemiol* **14**, 95-100.

139. Kesavachandran, C., Pathak, M.K., Fareed, M., Bihari, V., Mathur, N. and Srivastava, A.K. (2009) Health risks of employees working in pesticide retail shops: An exploratory study. *Indian J Occup Environ Med* **13**, 121-6.

140. Kesavachandran, C. and Mudiam, M.K.R. (2010) Adverse sub-clinical health effects in pesticide-exposed population: challenges and need for developing non-invasive clinical biomarkers. *Current Science* **99**, 1643

141. Kilburn, K.H. (1999) Evidence for chronic neurobehavioral impairment from chlorpyrifos an organophosphate insecticide (Dursban) used indoors. *Environ Epidemiol Toxicol* **1**, 153-162.

142. Kimura, J. (2006) Electrodiagnosis of the cranial nerves. *Acta Neurol Taiwan* **15**, 2-12.

143. Kimura, K., Yokoyama, K., Sato, H., Nordin, R.B., Naing, L., Kimura, S., Okabe, S., Maeno, T., Kobayashi, Y., Kitamura, F. and Araki, S. (2005) Effects of pesticides on the peripheral and central nervous system in tobacco farmers in Malaysia: studies on peripheral nerve conduction, brain-evoked potentials and computerized posturography. *Ind Health* **43**, 285-94.

144. Konieczny, B., Kossmann, S. and Makuch, M. (1999) Impaired respiratory muscle function in chemical plant workers producing chlorfenvinphos. *Ann Agric Environ Med* **6**, 21-5.

145. Langston, J.W., Ballard, P., Tetrud, J.W. and Irwin, I. (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979-80.

146. Lawlor, D.A., Day, I.N., Gaunt, T.R., Hinks, L.J., Timpson, N., Ebrahim, S. and Davey Smith, G. (2007) The association of the paraoxonase (PON1) Q192R polymorphism with depression in older women: findings from the British Women's Heart and Health Study. *J Epidemiol Community Health* **61**, 85-7.

147. Lee, B.W., London, L., Paulauskis, J., Myers, J. and Christiani, D.C. (2003) Association between human paraoxonase gene polymorphism and chronic symptoms in pesticide-exposed workers. *J Occup Environ Med* **45**, 118-22.

148. Lehman, R.M. (2004) A review of neurophysiological testing. *Neurosurg Focus* **16**, ECP1

149. Li, W.F., Costa, L.G., Richter, R.J., Hagen, T., Shih, D.M., Tward, A., Lusis, A.J. and Furlong, C.E. (2000) Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. *Pharmacogenetics* **10**, 767-79.

150. Little, S., Llewellyn, H. and Clarke, S.F. (2010) Sensory ataxia associated with chronic organophosphate pesticide exposure. *Hum Exp Toxicol* **29**, 689-93.

151. Loh, Y., Swanberg, M.M., Ingram, M.V. and Newmark, J. (2010) Case report: Long-term cognitive sequelae of sarin exposure. *Neurotoxicology* **31**, 244-6.

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

153. London, L., Bourne, D., Sayed, R. and Eastman, R. (2004) Guillain-Barre syndrome in a rural farming district in South Africa: a possible relationship to environmental organophosphate exposure. *Arch Environ Health* **59**, 575-80.

154. London, L., Flisher, A.J., Wesseling, C., Mergler, D. and Kromhout, H. (2005) Suicide and exposure to organophosphate insecticides: cause or effect? *Am J Ind Med* **47**, 308-21.

155. Lotti, M. (2002) Low-level exposures to organophosphorus esters and peripheral nerve function. *Muscle Nerve* **25**, 492-504.

156. Lotti, M. and Moretto, A. (2005) Organophosphate-induced delayed polyneuropathy. *Toxicol Rev* **24**, 37-49.

157. Lubis, S.H., El Sersi, M., Hussein, S.H., Sakian, N.I., Salleh, R., Rafai, J. and Al Khamesey, K. (2008) A biomarker for exposure to organophosphate and carbamate pesticides. *Asia Pac J Public Health* **20 Suppl**, 162-5.

158. Lundberg, I., Hogberg, M., Michelsen, H., Nise, G. and Hogstedt, C. (1997) Evaluation of the Q16 questionnaire on neurotoxic symptoms and a review of its use. *Occup Environ Med* **54**, 343-50.

159. Mackenzie Ross, S.J., Brewin, C.R., Curran, H.V., Furlong, C.E., Abraham-Smith, K.M. and Harrison, V. (2010) Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. *Neurotoxicol Teratol* **32**, 452-9.

160. Mackness, B., Durrington, P., Povey, A., Thomson, S., Dippnall, M., Mackness, M., Smith, T. and Cherry, N. (2003) Paraoxonase and susceptibility to organophosphorus poisoning in farmers dipping sheep. *Pharmacogenetics* **13**, 81-8.

161. Mann, C.J. (2003) Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emerg Med J* **20**, 54-60.

162. Manthripragada, A.D., Costello, S., Cockburn, M.G., Bronstein, J.M. and Ritz, B. (2010) Paraoxonase 1, agricultural organophosphate exposure, and Parkinson disease. *Epidemiology* **21**, 87-94.

163. Matmurodov, R.J., Khalimova, K.M. and Raimova, M.M. (2011) Polymorphism of the genes GSTM1, GSTT1, and environmental factors in the development of Parkinson's disease among representatives of the Uzbek nationality. *European Journal of Neurology* **18**, 501

164. McCauley, L.A., Joos, S.K., Lasarev, M.R., Storzbach, D. and Bourdette, D.N. (1999a) Gulf War unexplained illnesses: persistence and unexplained nature of self-reported symptoms. *Environ Res* **81**, 215-23.

165. McCauley, L.A., Joos, S.K., Spencer, P.S., Lasarev, M. and Shuell, T. (1999b) Strategies to assess validity of self-reported exposures during the Persian Gulf War. Portland Environmental Hazards Research Center. *Environ Res* **81**, 195-205.

166. McCauley, L.A., Rischitelli, G., Lambert, W.E., Lasarev, M., Sticker, D.L. and Spencer, P.S. (2001) Symptoms of Gulf War veterans possibly exposed to organophosphate chemical warfare agents at Khamisiyah, Iraq. *Int J Occup Environ Health* **7**, 79-89.

167. McCauley, L.A., Lasarev, M., Sticker, D., Rischitelli, D.G. and Spencer, P.S. (2002) Illness experience of Gulf War veterans possibly exposed to chemical warfare agents. *Am J Prev Med* **23**, 200-6.

168. McGuire, D., Garrison, L., Armon, C., Barohn, R., Bryan, W., Miller, R., Parry, G., Petajan, J. and Ross, M. (1996) Relationship of the Tufts Quantitative Neuromuscular Exam (TQNE) and the Sickness Impact Profile (SIP) in measuring progression of ALS. SSNJV/CNTF ALS Study Group. *Neurology* **46**, 1442-4.

169. McLeod, J.G. (1995) Investigation of peripheral neuropathy. *J Neurol Neurosurg Psychiatry* **58**, 274-83.

170. McNair, D., Lorr, M. and Droppleman, L. (1971) *EITS manual for the profile of mood states*, edn. San Diego: Educational and Industrial Testing Services.

171. Miranda, J., Lundberg, I., McConnell, R., Delgado, E., Cuadra, R., Torres, E., Wesseling, C. and Keifer, M. (2002a) Onset of grip- and pinch-strength impairment after acute poisonings with organophosphate insecticides. *Int J Occup Environ Health* **8**, 19-26.

172. Miranda, J., McConnell, R., Delgado, E., Cuadra, R., Keifer, M., Wesseling, C., Torres, E. and Lundberg, I. (2002b) Tactile vibration thresholds after acute poisonings with organophosphate insecticides. *Int J Occup Environ Health* **8**, 212-9.

173. Miranda, J., McConnell, R., Wesseling, C., Cuadra, R., Delgado, E., Torres, E., Keifer, M. and Lundberg, I. (2004) Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticides. *Occup Environ Med* **61**, e4

174. Misra, U.K., Prasad, M. and Pandey, C.M. (1994) A study of cognitive functions and event related potentials following organophosphate exposure. *Electromyogr Clin Neurophysiol* **34**, 197-203.

175. Misulis, K.E. and Head, T.C. (2003) *Essentials of clinical neurophysiology. 3rd Edition*, edn. Elsevier.

176. Mittal, T., Gupta, N., Kohli, A., Bhalla, A., Singh, B. and Singh, S. (2011) Correlation of defects in regional cerebral blood flow determined by 99mTc SPECT with residual neurocognitive testing abnormalities during and 3 months post exposure in acutely poisoned patients with organophosphates. *Clin Toxicol (Phila)* **49**, 464-70.

177. Miyaki, K., Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Yoshimura, K., Etoh, N., Matsumoto, Y., Kikuchi, Y., Kumagai, N. and Omae, K. (2005) Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J Occup Health* **47**, 299-304.

178. Morahan, J.M., Yu, B., Trent, R.J. and Pamphlett, R. (2007) A gene-environment study of the paraoxonase 1 gene and pesticides in amyotrophic lateral sclerosis. *Neurotoxicology* **28**, 532-40.

179. Muller-Vahl, K.R., Kolbe, H. and Dengler, R. (1999) Transient severe parkinsonism after acute organophosphate poisoning. *J Neurol Neurosurg Psychiatry* **66**, 253-4.

180. Murata, K., Araki, S., Yokoyama, K., Okumura, T., Ishimatsu, S., Takasu, N. and White, R.F. (1997) Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *J Neurol* **244**, 601-6.

181. Muttray, A., Spelmeyer, U., Degirmenci, M., Jung, D., Backer, G. and Hill, G. (2005) Acute effects of low doses of methyl parathion on human EEG. *Environ Toxicol Pharmacol* **19**, 477-483.

182. Nakajima, T., Ohta, S., Morita, H., Midorikawa, Y., Mimura, S. and Yanagisawa, N. (1998) Epidemiological study of sarin poisoning in Matsumoto City, Japan. *J Epidemiol* **8**, 33-41.

183. Nakajima, T., Ohta, S., Fukushima, Y. and Yanagisawa, N. (1999) Sequelae of sarin toxicity at one and three years after exposure in Matsumoto, Japan. *J Epidemiol* **9**, 337-43.

184. Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Minami, M. and Omae, K. (2001) Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. *Environ Health Perspect* **109**, 1169-73.

185. O'Leary, K.A., Edwards, R.J., Town, M.M. and Boobis, A.R. (2005) Genetic and other sources of variation in the activity of serum paraoxonase/diazoxonase in humans: consequences for risk from exposure to diazinon. *Pharmacogenet Genomics* **15**, 51-60.

186. Ohayo-Mitoko, G.J., Kromhout, H., Simwa, J.M., Boleij, J.S. and Heederik, D. (2000) Self reported symptoms and inhibition of acetylcholinesterase activity among Kenyan agricultural workers. *Occup Environ Med* **57**, 195-200.

187. Ohbu, S., Yamashina, A., Takasu, N., Yamaguchi, T., Murai, T., Nakano, K., Matsui, Y., Mikami, R., Sakurai, K. and Hinohara, S. (1997) Sarin poisoning on Tokyo subway. *South Med J* **90**, 587-93.

188. Ohtani, T., Iwanami, A., Kasai, K., Yamasue, H., Kato, T., Sasaki, T. and Kato, N. (2004) Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: a 5-year follow-up study. *Psychiatry Clin Neurosci* **58**, 624-9.

189. Okudera, H. (2002) Clinical features on nerve gas terrorism in Matsumoto. *J Clin Neurosci* **9**, 17-21.

190. Orozco-De Los Rios, I., Sanchez-Vizcaino, P.M., Gonzalez-Ramirez, D. and Garcia-Pinon, J. (2005) [Peripheral neuropathy and deterioration of the cognitive functions associated to chronic exposure to organophosphates]. *Rev Med Inst Mex Seguro Soc* **43**, 479-86.

191. Otero, G., Porcayo, R., Aguirre, D.M. and Pedrazza, M. (2000) Neurobehavioural study in workers exposed to pesticides. *Revista International de Contaminacion Ambiental* **16**, 67-74.

192. Ozturk, L., Izdes, S., Kesimci, E., Balikci, A.A. and Kanbak, O. (2008) Insecticide contamination as a cause of food related illness. *Clin Toxicol (Phila)* **46**, 917

193. Ozyurt, G., Kaya, F.N., Kahveci, F. and Alper, E. (2008) Comparison of SPECT findings and neuropsychological sequelae in carbon monoxide and organophosphate poisoning. *Clin Toxicol (Phila)* **46**, 218-21.

194. Page, W.F. (2003) Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. *Mil Med* **168**, 239-45.

195. Palacios-Nava, M.E., Paz-Roman, P., Hernandez-Robles, S. and Mendoza-Alvarado, L. (1999) [Persistent symptomatology in workers industrially exposed to organophosphate pesticides]. *Salud Publica Mex* **41**, 55-61.

196. Parron, T., Requena, M., Hernandez, A.F. and Alarcon, R. (2011) Association between environmental exposure to pesticides and neurodegenerative diseases. *Toxicol Appl Pharmacol* **256**, 379-85.

197. Pathak, M.K., Fareed, M., Bihari, V., Reddy, M.M.K., Patel, D.K., Mathur, N., Kuddus, M. and Kesavanchandran, C.N. (2011) Nerve conduction studies in sprayers occupationally exposed to mixture of pesticides in amango plantation at Lucknow, North India. *Toxicol Environ Chem* **93**, 188-196.

198. Payan-Renteria, R., Garibay-Chavez, G., Rangel-Ascencio, R., Preciado-Martinez, V., Munoz-Islas, L., Beltran-Miranda, C., Mena-Munguia, S., Jave-Suarez, L., Feria-Velasco, A. and De Celis, R. (2012) Effect of chronic pesticide exposure in farm workers of a Mexico community. *Arch Environ Occup Health* **67**, 22-30.

199. Peiris-John, R.J., Ruberu, D.K., Wickremasinghe, A.R., Smit, L.A. and van der Hoek, W. (2002) Effects of occupational exposure to organophosphate pesticides on nerve and neuromuscular function. *J Occup Environ Med* **44**, 352-7.

200. Pickett, W., King, W.D., Lees, R.E., Bienefeld, M., Morrison, H.I. and Brison, R.J. (1998) Suicide mortality and pesticide use among Canadian farmers. *Am J Ind Med* **34**, 364-72.

201. Picton, T.W. (1992) The P300 wave of the human event-related potential. *J Clin Neurophysiol* **9**, 456-79.

202. Pilkington, A. , Jamal, G.A., Gillham, R., Hansen, S., Buchanan, D., Kidd, M., Azis, M.A., Julu, P.O., Al-Rawas, S., Ballantyne, J.P., Hurley, J.F. and Soutar, C.A. (1999a) Epidemiological study of the relations 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 . *Edinburgh Institute of Occupational Medicine* **IOM report TM/99/02c**,

203. Pilkington, A. , Buchanan, D., Jamal, G.A., Kidd, M., Sewell, C., Donnan, P., Hansen, S., Tannahill, S.N., Robertson, A., Hurley, J.F. and Soutar, C.A. (1999b) Epidemiological study of the relationships between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy, and neuropsychological abnormalities in sheep farmers and dippers. Phase 2. Cross-sectional exposure-response study of sheep dippers. *Edinburgh Institute of Occupational Medicine* **IOM report TM/99/02b**,

204. Pilkington, A., Buchanan, D., Jamal, G.A., Gillham, R., Hansen, S., Kidd, M., Hurley, J.F. and Soutar, C.A. (2001) An epidemiological study of the relations between exposure to organophosphate pesticides and indices of chronic peripheral neuropathy and neuropsychological abnormalities in sheep farmers and dippers. *Occup Environ Med* **58**, 702-10.

205. Polich, J. and Kok, A. (1995) Cognitive and biological determinants of P300: an integrative review. *Biol Psychol* **41**, 103-46.

206. Povey, A.C., Mackness, M.I., Durrington, P.N., Dippnall, M., Smith, A.E., Mackness, B. and Cherry, N.M. (2005) Paraoxonase polymorphisms and self-reported chronic ill-health in farmers dipping sheep. *Occup Med (Lond)* **55**, 282-6.

207. Povey, A.C., Agius, R.M., McNamee, R., Burns, A., Creed, F. and Neary, D. (2007a) Disabling neuropsychiatric disease in farmers exposed to organophosphates: Phase 1: the study of health in agricultural work cohort. *Department for Environment Food and Rural Affairs* **Science and Research Project number VM02115**,

208. Povey, A.C., Jury, F., Dippnall, W.M., Smith, A.E., Thomson, S., Mackness, B., Mackness, M., Durrington, P. and Cherry, N.M. (2007b) GST CYP and PON1 polymorphisms in farmers attributing ill health to organophosphate-containing sheep dip. *Biomarkers* **12**, 188-202.

209. Povey, A.C., Agius, R.M., McNamee, R., Burns, A., Creed, F. and Neary, D. (2009) Disabling neuropsychiatric disease in farmers exposed to organophosphates: Phase 2 study. *Department for Environment Food and Rural Affairs* **Science and Research Project number VM02115**,

210. Povey, A.C., Rees, H.G., Thompson, J.P. and Karalleidde, L. (2010) Prospective cohort study of sheep dip exposure and 'dippers' flu' (RR775). *HSE Books*

211. Povey, A.C. (2010) Gene-environmental interactions and organophosphate toxicity. *Toxicology* **278**, 294-304.

212. Proctor, S.P., Heaton, K.J., Heeren, T. and White, R.F. (2006) Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. *Neurotoxicology* **27**, 931-9.

213. Radloff, L.S. (1977) The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement* **1**, 385-401.

214. Rainier, S., Bui, M., Mark, E., Thomas, D., Tokarz, D., Ming, L., Delaney, C., Richardson, R.J., Albers, J.W., Matsunami, N., Stevens, J., Coon, H., Leppert, M. and Fink, J.K. (2008) Neuropathy target esterase gene mutations cause motor neuron disease. *Am J Hum Genet* **82**, 780-5.

215. Ray, D.E. and Richards, P.G. (2001) The potential for toxic effects of chronic, low-dose exposure to organophosphates. *Toxicol Lett* **120**, 343-51.

216. Rees, H. (2011) Sheep dipping related treatments and 'Dippers' Flu'. *MD Thesis submitted to the University of Wales*

217. Reeves, A.G. and Swenson, R.S. (2008) *Disorders of the nervous system: a primer*, edn. UK: Dartmouth Medical School.

218. Rehner, T.A., Kolbo, J.R., Trump, R., Smith, C. and Reid, D. (2000) Depression among victims of south Mississippi's methyl parathion disaster. *Health Soc Work* **25**, 33-40.

219. Reidy, T.J., Bowler, R.M., Rauch, S.S. and Pedroza, G.I. (1992) Pesticide exposure and neuropsychological impairment in migrant farm workers. *Arch Clin Neuropsychol* **7**, 85-95.

220. Rice, N.E., Bandinelli, S., Corsi, A.M., Ferrucci, L., Guralnik, J.M., Miller, M.A., Kumari, M., Murray, A., Frayling, T.M. and Melzer, D. (2009) The paraoxonase (PON1) Q192R polymorphism is not associated with poor health status or depression in the ELSA or INCHIANTI studies. *Int J Epidemiol* **38**, 1374-9.

221. Richter, R.J., Jarvik, G.P. and Furlong, C.E. (2009) Paraoxonase 1 (PON1) status and substrate hydrolysis. *Toxicol Appl Pharmacol* **235**, 1-9.

222. Riddle, J.R., Brown, M., Smith, T., Ritchie, E.C., Brix, K.A. and Romano, J. (2003) Chemical warfare and the Gulf War: a review of the impact on Gulf veterans' health. *Mil Med* **168**, 606-13.

223. Rohlman, D.S., Lasarev, M., Anger, W.K., Scherer, J., Stupfel, J. and McCauley, L. (2007) Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology* **28**, 374-80.

224. Rohlman, D.S., Anger, W.K. and Lein, P.J. (2011) Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure. *Neurotoxicology* **32**, 268-76.

225. Roldan-Tapia, L., Parron, T. and Sanchez-Santed, F. (2005) Neuropsychological effects of long-term exposure to organophosphate pesticides. *Neurotoxicol Teratol* **27**, 259-66.

226. Roldan-Tapia, L., Nieto-Escamez, F.A., del Aguila, E.M., Laynez, F., Parron, T. and Sanchez-Santed, F. (2006) Neuropsychological sequelae from acute

poisoning and long-term exposure to carbamate and organophosphate pesticides. *Neurotoxicol Teratol* **28**, 694-703.

227. Rothlein, J., Rohlman, D., Lasarev, M., Phillips, J., Muniz, J. and McCauley, L. (2006) Organophosphate pesticide exposure and neurobehavioral performance in agricultural and non-agricultural Hispanic workers. *Environ Health Perspect* **114**, 691-6.

228. Rubin, M. (2008) Peripheral nervous system and motor unit disorders. In: *The Merck Manuals 19th Edition*, Merck Sharpe & Dohme Corp]

229. Salvi, R.M., Lara, D.R., Ghisolfi, E.S., Portela, L.V., Dias, R.D. and Souza, D.O. (2003) Neuropsychiatric evaluation in subjects chronically exposed to organophosphate pesticides. *Toxicol Sci* **72**, 267-71.

230. Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A. and Burcar, P.J. (1988) Chronic neurological sequelae of acute organophosphate pesticide poisoning. *Arch Environ Health* **43**, 38-45.

231. Savy, C.Y., Morris, C.M., Blain, P.G. and Judge, S.J. (2011) Behavioural effects of low level exposure to organophosphate pesticides. *Toxicology* **290**, 116

232. Sedziak, R., Kazibutowska, Z. and Kossmann, S. (1998) Analysis of EEG frequency in workers employed in the departmental producing chlorfenvinphos . *Medycyna Pracy* **49**, 113-117.

233. Shahar, E. and Andraws, J. (2001) Extra-pyramidal parkinsonism complicating organophosphate insecticide poisoning. *Eur J Paediatr Neurol* **5**, 261-4.

234. Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R. and Dunbar, G.C. (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* **59 Suppl 20**, 22-33;quiz 34-57.

235. Siao, P. and Cros, D.P. (2003) Quantitative sensory testing. *Phys Med Rehabil Clin N Am* **14**, 261-86.

236. Solomon, C., Poole, J., Palmer, K.T., Peveler, R. and Coggon, D. (2007) Neuropsychiatric symptoms in past users of sheep dip and other pesticides. *Occup Environ Med* **64**, 259-66.

237. Sosan, M.B., Akingbohungbe, A.E., Durosinmi, M.A. and Ojo, I.A. (2010) Erythrocyte cholinesterase enzyme activity and hemoglobin values in cacao farmers of southwestern Nigeria as related to insecticide exposure. *Arch Environ Occup Health* **65**, 27-33.

238. Soummer, A., Megarbane, B., Boroli, F., Arbelot, C., Saleh, M., Moesch, C., Fournier, E. and Rouby, J.J. (2011) Severe and prolonged neurologic toxicity following subcutaneous chlorpyrifos self-administration: a case report. *Clin Toxicol (Phila)* **49**, 124-7.

239. Spencer, P.S. (2001) Epidemiological and Clinical Evidence for Residual Organophosphate Neurotoxicity. *Govt Reports Announcements & Index* **13**,

240. Spielberger, C.D., Gorsuch, R.L., Lushene, P.R., Vagg, P.R. and Jacobs, A.G. (1983) *Manual for the State - Trait Anxiety Inventory (Form Y)*, edn. Palo Alto: Consulting Psychologists Pres Inc.

241. Spitzer, R.L., Williams, J.B., Gibbon, M. and First, M.B. (1992) The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. *Arch Gen Psychiatry* **49**, 624-9.

242. Srinivasan, K.G., Praveen Kumar, M., Ushanandhini, K.P. and Ramprabananth, S. (2010) MRI eye-of-the-tiger sign in organophosphate poisoning: a case report. *Neuroradiology Journal* **23**, 407-411.

243. Srivastava, A.K., Gupta, B.N., Bihari, V., Mathur, N., Srivastava, L.P., Pangtey, B.S., Bharti, R.S. and Kumar, P. (2000) Clinical, biochemical and neurobehavioural studies of workers engaged in the manufacture of quinalphos. *Food Chem Toxicol* **38**, 65-9.

244. Stallones, L. and Beseler, C. (2002a) Pesticide poisoning and depressive symptoms among farm residents. *Ann Epidemiol* **12**, 389-94.

245. Stallones, L. and Beseler, C. (2002b) Pesticide illness, farm practices, and neurological symptoms among farm residents in Colorado. *Environ Res* **90**, 89-97.

246. Starks, S.E., Gerr, F., Kamel, F., Lynch, C.F., Jones, M.P., Alavanja, M.C., Sandler, D.P. and Hoppin, J.A. (2012a) Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the Agricultural Health Study. *Neurotoxicol Teratol* **34**, 168-76.

247. Starks, S.E., Hoppin, J.A., Kamel, F., Lynch, C.F., Jones, M.P., Alavanja, M.C., Sandler, D.P. and Gerr, F. (2012b) Peripheral nervous system function and organophosphate pesticide use among licensed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect* **120**, 515-20.

248. Steenland, K., Jenkins, B., Ames, R.G., O'Malley, M., Chrislip, D. and Russo, J. (1994) Chronic neurological sequelae to organophosphate pesticide poisoning. *Am J Public Health* **84**, 731-6.

249. Steenland, K., Dick, R.B., Howell, R.J., Chrislip, D.W., Hines, C.J., Reid, T.M., Lehman, E., Laber, P., Krieg, E.F. Jr and Knott, C. (2000) Neurologic function among termiticide applicators exposed to chlorpyrifos. *Environ Health Perspect* **108**, 293-300.

250. Stephens, R., Spurgeon, A., Calvert, I.A., Beach, J., Levy, L.S., Berry, H. and Harrington, J.M. (1995) Neuropsychological effects of long-term exposure to organophosphates in sheep dip. *Lancet* **345**, 1135-9.

251. Stephens, R. and Sreenivasan, B. (2004) Neuropsychological effects of long-term low-level organophosphate exposure in orchard sprayers in England. *Arch Environ Health* **59**, 566-74.

252. Storzbach, D., Campbell, K.A., Binder, L.M., McCauley, L., Anger, W.K., Rohlman, D.S. and Kovera, C.A. (2000) Psychological differences between veterans with and without Gulf War unexplained symptoms. Portland Environmental Hazards Research Center. *Psychosom Med* **62**, 726-35.

253. Sylos Labini, F., Meli, A., Tufarelli, D. and Colosimo, A. (2008) A new set of paramters for computerised dynamic posturography. *Biophysics and Bioengineering Lett* **1**, 1-9.

254. Tahmaz, N., Soutar, A. and Cherrie, J.W. (2003) Chronic fatigue and organophosphate pesticides in sheep farming: a retrospective study amongst people reporting to a UK pharmacovigilance scheme. *Ann Occup Hyg* **47**, 261-7.

255. Teixeira, C.F., Augusto, L.G. and Morata, T.C. (2003) [Hearing health of workers exposed to noise and insecticides]. *Rev Saude Publica* **37**, 417-23.

256. Teng, E.L. and Chui, H.C. (1987) The Modified Mini-Mental State (3MS) examination. *J Clin Psychiatry* **48**, 314-8.

257. Tochigi, M., Umekage, T., Otani, T., Kato, T., Iwanami, A., Asukai, N., Sasaki, T. and Kato, N. (2002) Serum cholesterol, uric acid and cholinesterase in victims of the Tokyo subway sarin poisoning: a relation with post-traumatic stress disorder. *Neurosci Res* **44**, 267-72.

258. Tochigi, M., Otani, T., Yamasue, H., Kasai, K., Kato, T., Iwanami, A., Kato, N. and Sasaki, T. (2005) Support for relationship between serum cholinesterase and post-traumatic stress disorder; 5-year follow-ups of victims of the Tokyo subway sarin poisoning. *Neurosci Res* **52**, 129-31.

259. Turlough FitzGerald, M.J., Greuner, G. and Mtui, E. (2009) *Clinical neuroanatomy and neuroscience. 5th Edition*, edn. Elsevier.

260. van der Mark, M., Brouwer, M., Kromhout, H., Nijssen, P., Huss, A. and Vermeulen, R. (2012) Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. *Environ Health Perspect* **120**, 340-7.

261. Wang, A.G., Liu, R.S., Liu, J.H., Teng, M.M. and Yen, M.Y. (1999) Positron emission tomography scan in cortical visual loss in patients with organophosphate intoxication. *Ophthalmology* **106**, 1287-91.

262. Waxman, S.J., Kocsis, J.D. and Stys, P.K. (1995) *The axon: structure, function and pathophysiology*, edn. UK: Oxford University Press.

263. Weimer, L.H. (2010) Autonomic testing: common techniques and clinical applications. *Neurologist* **16**, 215-22.

264. Wesseling, C., Keifer, M., Ahlbom, A., McConnell, R., Moon, J.D., Rosenstock, L. and Hogstedt, C. (2002) Long-term neurobehavioral effects of mild poisonings with organophosphate and n-methyl carbamate pesticides among banana workers. *Int J Occup Environ Health* **8**, 27-34.

265. Wesseling, C., van Wendel de Joode, B., Keifer, M., London, L., Mergler, D. and Stallones, L. (2010) Symptoms of psychological distress and suicidal ideation among banana workers with a history of poisoning by organophosphate or n-methyl carbamate pesticides. *Occup Environ Med* **67**, 778-84.

266. White, R.F., Proctor, S.P., Heeren, T., Wolfe, J., Krengel, M., Vasterling, J., Lindem, K., Heaton, K.J., Sutker, P. and Ozonoff, D.M. (2001) Neuropsychological function in Gulf War veterans: relationships to self-reported toxicant exposures. *Am J Ind Med* **40**, 42-54.

267. Wills, A.M., Landers, J.E., Zhang, H., Richter, R.J., Caraganis, A.J., Cudkowicz, M.E., Furlong, C.E. and Brown, R.H. Jr (2008) Paraoxonase 1 (PON1) organophosphate hydrolysis is not reduced in ALS. *Neurology* **70**, 929-34.

268. Wolfe, J., Brown, P.J. and Kelley, J.M. (1993) Reassessing war stress: exposure and the Persian Gulf War. *J Soc Issues* **49**, 15-31.

269. Wolfe, J., Proctor, S.P., Davis, J.D., Borgos, M.S. and Friedman, M.J. (1998) Health symptoms reported by Persian Gulf War veterans two years after return. *Am J Ind Med* **33**, 104-13.

270. Woodward, S.H., Kaloupek, D.G., Streeter, C.C., Martinez, C., Schaer, M. and Eliez, S. (2006) Decreased anterior cingulate volume in combat-related PTSD. *Biol Psychiatry* **59**, 582-7.

271. Yamasue, H., Kasai, K., Iwanami, A., Ohtani, T., Yamada, H., Abe, O., Kuroki, N., Fukuda, R., Tochigi, M., Furukawa, S., Sadamatsu, M., Sasaki, T., Aoki, S., Ohtomo, K., Asukai, N. and Kato, N. (2003) Voxel-based analysis of MRI reveals anterior cingulate gray-matter volume reduction in posttraumatic stress disorder due to terrorism. *Proc Natl Acad Sci U S A* **100**, 9039-43.

272. Yamasue, H., Abe, O., Kasai, K., Suga, M., Iwanami, A., Yamada, H., Tochigi, M., Ohtani, T., Rogers, M.A., Sasaki, T., Aoki, S., Kato, T. and Kato, N. (2007) Human brain structural change related to acute single exposure to sarin. *Ann Neurol* **61**, 37-46.

273. Yan, C., Tan, D. and Peng, S. (2011) Progress in cognitive impairment induced by low-dose exposure to organophosphate pesticides. *Chinese Journal of Pharmacology and Toxicology* **25**, 397-401.

274. Yanagisawa, N., Morita, H. and Nakajima, T. (2006) Sarin experiences in Japan: acute toxicity and long-term effects. *J Neurol Sci* **249**, 76-85.

275. Yao, X. and Zhou, Z. (2008) Progress of studies on human health impacts of long-term exposure to organophosphorous pesticides. *J Environ Occ Med* **25**, 409-411.

276. Yokoyama, K. (2007) Our recent experiences with sarin poisoning cases in Japan and pesticide users with references to some selected chemicals. *Neurotoxicology* **28**, 364-73.

277. Zigmond, A.S. and Snaith, R.P. (1983) The hospital anxiety and depression scale. *Acta Psychiatr Scand* **67**, 361-70.

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

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HPA COT Secretariat

August 2012

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EXCERPTS FROM MINUTES OF COT MEETINGS HELD IN

- **SEPTEMBER 2007, (TOX/MIN/2007/05)**

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- **SEPTEMBER 2009 (TOX/MIN/2009/05)**

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- **DECEMBER 2009,(TOX/MIN/2009/07)**

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LETTER ADDRESSED TO THE COT

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