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Neurobehavioural problems following low level exposure to organophosphate pesticides: A systematic & meta-analytic review.

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ABSTRACT

Meta-analysis was carried out to determine the neurotoxic effects of long term exposure to low levels of organophosphates (OPs). Concern about the effects of OPs on human health has been growing as they are increasingly used throughout the world for a variety of agricultural, industrial and domestic purposes. The neurotoxic effects of acute poisoning are well established but the possibility that low level exposure causes ill health is controversial. It is important to get a clear answer to this question as more individuals are at risk of low level exposure than acute poisoning. Although a number of reviews on this topic have been published in the past, authors have come to conflicting conclusions. To date, none of these reviews have attempted quantitative evaluation of study findings using meta-analysis. This paper reviews the available evidence concerning the neurotoxicity of low level exposure to OPs and goes on to report the results of a meta-analysis of 14 studies which fulfilled criteria for this type of statistical analysis (means and standard deviations of dependant variables reported). Data were assimilated from more than 1600 participants. The majority of well designed studies found a significant association between low level exposure to OPs and impaired neurobehavioural function which is consistent, small to moderate in magnitude and concerned primarily with cognitive functions such as psychomotor speed, executive function, visuo-spatial ability, working and visual memory. Unresolved issues in the literature which should become the focus of further studies are highlighted and discussed.

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1. Background

Pesticides prevent millions of people from starving to death and from disease, but they are harmful to humans under certain circumstances. Organophosphate pesticides are the most widely used insecticides in the world and are considered by the World Health Organisation to be one of the most hazardous pesticides to vertebrate animals, responsible for many cases of poisoning worldwide, particularly in developing countries where adequate protective measures are lacking (De Silva et al, 2006; WHO report 1990). Concern about the effects of organophosphates (OPs) on human health has been growing as they are increasingly used throughout the world for a variety of agricultural, domestic and industrial purposes. For example, they have been used in agriculture and horticulture pesticides; in veterinary medicines to prevent ectoparasitic infections of farm animals and domestic pets; some human medicines (e.g. to treat head lice); and in public hygiene products both for use by professional operators and the general public to control insect infestations in public and residential buildings, outside spaces and gardens; and OPs are used in industry as lubricants, plasticisers and flame retardants (COT report, 1999; Karalliedde, et al, 2001; Mackenzie Ross et al, 2011).

The neurotoxic effects of high level acute poisoning are well established and involve inhibition of the enzyme acetylcholinesterase (AChE) causing changes in peripheral, autonomic and central nervous system function (the cholinergic crisis). However, the possibility that long-term low-level exposure to OPs in doses below that causing acute toxicity causes ill health is controversial.

A number of researchers have addressed this question using a variety of different methodologies and populations, but previous research has produced inconsistent findings, with some studies finding evidence of ill health and cognitive impairment following low level

organophosphate exposure while others have not (see reviews by Alavanja et al 2004; Arcury & Quandt 1998; Colosio et al, 2003; COT Report 1999; De Silva et al 2006; ECETOC Report 1998; Kamel & Hoppin 2004; Mearns et al, 1994; Ontario College of Family Physicians (OCFP) Report 2004; Ray, 1998a; 1998b; Royal Colleges' Report, 1998). Major methodological differences may account for these inconsistencies such as examination of different occupational groups with different levels and routes of exposure, use of protective clothing, cohorts from different cultural backgrounds examined over different time periods (e.g. following a single episode of exposure, several years of exposure or over a lifetime).

Since many more individuals are likely to be at risk of long-term, low level exposure, rather than acute poisoning it is important to get a clear answer to the question of whether low level exposure is harmful to human health. The aim of this paper is to review the available evidence concerning the neurotoxicity of long-term, low level exposure to organophosphate pesticides. In this review, long term, low level exposure to OPs is defined as 'repeated or prolonged exposure to doses which do not produce recognised clinical symptoms of acute toxicity requiring medical evaluation or intervention'. As mentioned earlier, the neurotoxic effects of high level acute poisoning are well established and can result in damage to the peripheral, autonomic and central nervous system (COT report, 1999), but the evidence concerning the neurotoxicity of repeated low level exposure to OPs is equivocal.

This review will focus on the effects of low level exposure to OPs on neurobehavioural function and will identify and evaluate studies which include neuropsychological assessment of study participants. Neuropsychology is a discipline which has an important role to play in the evaluation of toxic substances. It has been described as the most sensitive means of examining the effects of toxic exposure as neuropsychological testing is capable of detecting signs of neurotoxic damage in the absence of other

neurological signs (Berent & Albers, 2005; Hartman, 1995; Lezak, et al, 2004). Neuropsychological assessment involves the use of objective, standardised psychometric tests which measure and quantify aspects of psychological functioning such as intellectual level, memory, attention, language, planning, visuo-spatial and verbal reasoning. These tests have the advantage of being relatively inexpensive, non-invasive and portable. They have known reliability and validity and an individuals' test performance can be compared to that derived from other population samples, thus aiding interpretation of the data. Furthermore, the results of neuropsychological testing are generally considered more valid than information obtained via self-report as individuals may lack awareness or insight into their difficulties or perceive them to be worse than they are in reality (this is particularly true of patients suffering from depression and/or anxiety: Lezak, 2004; Bruce et al, 2009). Subjective symptom reporting of cognitive difficulties does not always correlate well with actual performance on psychometric tests and so studies which relied exclusively on questionnaire measures of neurobehavioural function were excluded from this review. The only exception to this rule were studies which focussed on mood state rather than cognitive function, provided they used questionnaire measures with accepted reliability, validity, sensitivity and specificity in terms of screening for psychiatric disorders, (e.g. the General Health Questionnaire), 2. Methods

We identified epidemiologic studies published between 1960 and 10th February 2012 concerning the neurotoxicity of long-term, low level exposure to organophosphate pesticides. Studies were located by searching computerised databases including Medline, Embase and Psycinfo and both subject headings and textword search strategies were used. Government working party reports, relevant textbooks and references cited at the end of articles were also examined to ensure all relevant material was included in this review.

2.1 Criteria for considering studies for this review

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

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

INSERT TABLE 1 HERE

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

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

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

Does the study evaluate the effects of exposure to organophosphates or does it concern exposure to a mixture of pesticides, including OPs?

Does the study evaluate the effects of long-term, low level exposure to OPs in the absence of a history of acute exposure?

Does the study include participants with a history of acute exposure? If so, do they take this into account in their analysis by analysing these individuals as a separate group?

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

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

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

3. Results

3.1 Numbers of articles retrieved from database searches

A total of 644 articles from the three databases were identified as potentially relevant by the three databases. The titles and abstracts of these articles were subsequently reviewed and assessed for eligibility according to the inclusion/exclusion criteria in Table 1. After duplicates were removed, a sample of 38 relevant articles remained. In depth inspection of these articles and their references identified a further 7 studies which had not been identified by the database searches, but which met the inclusion criteria for this review. This left a final sample of 45 original articles for review.

3.2 Excluded studies

The first step of the review process was to determine whether all 45 articles selected from the initial screening of titles and abstracts, met inclusion criteria for this review. This was not always apparent from a review of titles and abstracts. Seventeen studies were excluded following this second stage of the review because they did not meet the inclusion criteria listed in Table 1. For example, outcome measures used in eight studies involved subjective symptom questionnaires rather than objective neurobehavioural measures (Ahmed & Davies, 1997; Ciesielski et al, 1994; Cox et al, 2005; Davies et al, 1999, Kamel et al, 2007, Ohayo-Mitoko et al, 2000; Smit et al, 2003; Solomon et al, 2007) and in another study individuals underwent a neurological examination rather than a neuropsychological assessment (Beach et al, 1996). An additional study was excluded because it did not evaluate the effects of low-level exposure on neurobehavioural functioning, but rather whether symptom reporting at time of exposure predicted subsequent performance on neuropsychological tests (Stephens et al, 1996). Seven studies failed to provide adequate information about exposure history (Bosma et al, 2000; Dimich-Ward et al, 1996; Kilburn, 1999; Korsak & Sato, 1977; Kurlycheck & Morrow, 1989; Richter et al, 1992; Starks et al, 2012) and this included a

recent study by Starks et al (2012) of 701 licensed pesticide applicators enrolled in the Agricultural Health Study in the USA. The primary reason for excluding this study was failure to examine a suitable, matched comparison group of unexposed individuals (97% of study participants reported using OPs); but Starks et al also failed to provide reliable exposure information Lifetime exposure history was estimated by integrating data collected at three different time points during the Agricultural Health Study (e.g. at enrolment, 5 and 10 year follow) and assuming frequency and duration of pesticide use remained consistent in between these time points. The reliability of such an assumption is open to question and thus the exposure metrics may be invalid. Furthermore, associations between pesticide use and neurobehavioural function were estimated with linear regression, but the authors controlled for a vast number of potentially confounding variables (height, education, smoking, alcohol and caffeine consumption, mood, medication, exposure to other potentially neurotoxic substances, head injury), including age which is inextricably linked with duration of exposure; and reading ability which may be adversely affected by exposure to OPs (Mackenzie Ross et al 2007). Statistical control of so many variables, some of which are inextricably linked to the variables of interest, reduces the likelihood of finding meaningful associations between exposure metrics and neurobehavioural test performance.

A further twelve studies were excluded because the study design did not adequately address the question of whether long-term, low level exposure to OPs impaired neurobehavioural function. The literature concerning this issue encompasses considerable variation in study methodology. It is possible to group studies according to design and three broad study designs are apparent in the literature; (1) epidemiological studies which use proxy measures of exposure such as occupational group (2) pre/post episode or season of exposure evaluations (3) epidemiological studies which provide quantitative information about exposure history. However the first two study designs do not adequately address the issue of

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

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

However, most studies failed to address the issue of whether long-term, low level exposure to OPs causes ill health and were therefore outside the scope of this review. The exception is the

study by Bazlewicz-Walczak et al (1999) in which two types of analyses were undertaken, both pre and post season evaluations looking for change in performance over time, but also comparisons of exposed and unexposed cohorts prior to the spraying season, matched on important variables which might otherwise affect cognitive function. The latter analysis is crucial for establishing whether cumulative, low level exposure is the causative factor, since any cohort comparisons undertaken following spraying seasons may simply pick up immediate, acute effects of exposure. This study was therefore retained in the meta-analysis.

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

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

This review will now describe these studies. They will be grouped according to the occupational status of study participants and country of origin because level and route of exposure varied between jobs and in developing and developed nations. Details are also provided concerning the neurobehavioural measures used in the study and whether the control subjects were matched on important variables, known to affect performance on cognitive tests, such as age and years of education. All of this information is summarised in Tables 2.

3.3.1 Chemical plant manufacturers

Developing countries

Srivastava et al (2000) examined 59 Indian workers exposed to different chemicals during the manufacture of 'quinalphos'. Exposed subjects had been employed in the manufacture of 'quinalphos' for an average of 5 years and were working in the production unit at the time of assessment. They were examined 5-6 hours after a shift. Their performance on neurobehavioural tests was compared with that of 17 control subjects who were not engaged in the manufacture or handling of quinalphos (i.e. tea vendors, road side hawkers) Groups were matched for age and sex, but controls were more educated than exposed subjects. All participants underwent a general medical examination, blood tests to assess recent exposure and psychometric testing. Although mean blood AChE levels in the exposed and control groups were not significantly different, exposed subjects reported more symptoms of fatigue and weakness; had a higher prevalence of abnormal plantar and ankle reflex; and lower scores on digit span, digit symbol and Bourdon Weirisma vigilance test. None had a history of acute OP poisoning over the preceding years. The authors conclude that chronic low dose exposure to OPs can cause nervous system damage and that AChE monitoring of chemical plant workers may not be adequate, because OPs may inhibit enzymes other than cholinesterase. The main limitation of this study is the fact that the control group was not matched to the exposed group for level of education and would be expected to outperform the exposed cohort. A further criticism concerns the limited amount of information provided about exposure history.

Amr et al (1997) examined 208 Egyptian pesticide formulators, 172 pesticide applicators and compared them to 233 controls (72 from an urban textile factory and 151 from a rural area, matched to exposed subjects for age, social class and education). Formulators and Applicators had been exposed to a range of pesticides (including OPs, organochlorines, carbamates and synthetic pyrethroids) for at least 2 years. All study participants were

working when the study was undertaken and were assessed by a psychiatrist with reference to the American Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R; American Psychiatric Association, 1994) and completed the General Health Questionnaire. Psychiatric disorders were significantly higher among pesticide formulators and applicators than controls and in those with a longer duration of exposure (e.g. more than 20 years). Furthermore, the incidence of reactive depression was nearly equal in all groups, but the incidence of neurotic or dysthymic disorder was higher in exposed subjects than in controls and higher than that seen in the general population of Egypt. The authors conclude that the increase in psychiatric morbidity relates to the cholinergic effects of pesticides. A major weakness of this study is the failure to provide any information about exposure history, other than to describe the exposed subjects as having had heavy and continuous exposure. It is impossible to determine whether they have a history of acute poisoning. Furthermore, the authors missed an opportunity to compare applicators with formulators directly. Working practices, use of protective clothing and routes of exposure may have differed in these groups.

3.3.2 *Pest control operators*

Developed countries

Steenland et al (2000) looked at the effects of low-level exposure to an OP pesticide called 'chlorpyrifos' by examining 191 termiticide applicators who had applied this pesticide for an average of 1.8 years (median 2.4 years; 67% had applied pesticides in the last year), with 189 non exposed controls. Half of the control cohort were recruited from lists of blue collar state employees such as maintenance workers and corrections officers whilst the remainder were friends of the exposed subjects. Exposed and unexposed groups were matched for age and sex, but controls were more educated than exposed subjects. All participants underwent an extensive range of tests including clinical examination, urine and blood tests to assess recent

exposure and genotype (in regard to paraoxonase), vibrotactile sensitivity, postural sway, manual dexterity, eye-hand coordination, arm/hand tremor, vision and olfaction tests, nerve conduction velocity and cognitive function. The exposed subjects reported more symptoms including memory problems, emotional states, fatigue and loss of muscle strength, but few significant differences were found on quantitative tests. The exposed subjects performed more poorly than controls on pegboard turning tests and some postural sway tests, but there were no significant differences between the exposed and nonexposed groups on most of the cognitive tests. Eight study participants reported a past history of acute poisoning, but only one sought medical help. These men showed a pattern of worse performance on a range of tests including simple RT and continuous performance, when compared to other applicators. The authors concluded that increased symptom reporting in the exposed group was cause for concern, that their neurologic tests may not have been sensitive enough to detect some of the effects of exposure and that there is evidence for delayed effects in subjects with a history of poisoning. The main limitation of this study is the fact that study participants had a relatively short history of exposure to OPs.

3.3.3 Farm workers & pesticide applicators

Developed countries

Rodnitzky et al (1975) studied 23 farmers and commercial pesticide applicators in Iowa who regularly used OP compounds (and had done so within 2 weeks of testing), but were asymptomatic and compared them with 23 non-exposed farmers. Mean plasma AChE levels were within normal limits (but slightly lower in exposed farmers) but the groups did not differ significantly on tests of memory or reaction time (RT). However, applicators had higher levels of anxiety. Limitations of this study include the possibility that the control group, who were also farmers, had significant levels of exposure to OPs in the past and

lifetime exposure history of study participants was not provided and the sample size is very small.

Ames et al (1995) examined 45 Californian pesticide applicators with a prior history of documented cholinesterase inhibition (according to medical supervision records), but with no clinical symptoms of acute poisoning and compared them to controls. Pesticide applicators were asked to bring a friend of comparable age, who had not been exposed to pesticides, to act as a control subject. Exposed and unexposed groups were not matched for age or education, the exposed cohort being older and less educated. Subjects underwent nerve conduction studies, vibrotactile sensitivity tests, a test of postural sway and eight neuropsychological tests of psychomotor speed, attention, fine motor control, memory and mood state. No evidence of neurobehavioural problems was found in the exposed cohort and the authors conclude that neurological sequelae can be prevented by avoiding acute poisoning. However, no information is provided about exposure history other than the fact workers had been exposed to cholinesterase inhibiting pesticides and the duration of time workers were exposed to pesticides is unclear.

Developing countries

Farahat et al (2003) examined 52 Egyptian pesticide applicators during the spraying season and compared them to 50 non-exposed controls who were clerks and administrators employed by the Ministry of Agriculture (matched for age, years of education and social class). None of the applicators reported an incident of acute poisoning which led to hospitalization. All participants underwent a clinical examination, blood tests to assess recent exposure and psychometric testing. The mean level of serum AChE was significantly lower in exposed subjects but within normal limits and did not relate to performance on psychometric tests. After adjusting for potentially confounding factors (age and education)

the performance of the exposed subjects was significantly lower on similarities, digit symbol, digit span, Trails A and B, letter cancellation and the Benton visual retention test (Benton et al, 1994). This was related to duration of exposure. The authors conclude that the effects of low to moderate exposure to OPs over a prolonged period of time (10-20 years) may be more wide ranging than previously realized, that workers can exhibit mild symptoms of intoxication without any change in blood AChE activity and that psychometric assessment is a useful method for the early detection of chronic effects of OP pesticide exposure.

3.3.4 *Fruit Tree Sprayers*

Developed countries

Stephens and Sreenivasan (2004) looked at the neuropsychological effects of long-term low level exposure to OPs in 37 English orchard sprayers, exposed to OPs for an average of 14 years, none of whom had a history of acute poisoning. Their performance on 7 neuropsychological tests was compared with 26 pig farmers and 31 construction workers, matched for age and education. A period of 2 months was imposed between any use of an OP pesticide and neurobehavioural testing to ensure the absence of acute effects. Pig farmers had a history of exposure to pesticides. Orchard sprayers (and pig farmers) differed from unexposed construction workers in terms of the time taken to complete negative statements of the ACTS syntactic reasoning test. However, psychometric test findings did not correlate with the index of cumulative exposure used in this study, but the authors suggest this may be due to measurement error inherent in the index.

Fiedler et al (1997) compared 57 fruit tree sprayers in New Jersey who had spent an average of 27 years farming (with no history of acute poisoning resulting in hospitalisation) with unexposed controls who comprised blueberry/cranberry growers and hardware store owners from the same community. Groups were matched for age, but controls were more educated

and had higher reading scores than the exposed subjects. Examiners were blind to group membership at time of neuropsychological testing. The exposed cohort had slower reaction time (although age predicted some of the variance in RT scores), but no other differences between the groups on neuropsychological testing were found. However, Fiedler et al corrected their data for the influence of reading scores, used to assess premorbid IQ. This may have confounded the results as reading scores may be affected by exposure to OPs.

Developing countries

London et al (1997) looked at the neurobehavioural effects of long-term, low level exposure to OPs by examining 163 African fruit tree sprayers who had been employed in agricultural work for an average of 19 years and comparing them with 84 unexposed labourers. Groups were matched for age, years in education and levels of illiteracy. Neuropsychological tests had to be adapted for the study population due to cultural differences reported by previous studies which influence performance on standard tests and because participants had little formal education. Nine percent of exposed subjects had a history of acute poisoning with OPs and over eleven percent had a history of exposure to other neurotoxic chemicals. Alcohol consumption was high. Nine controls had a history of pesticide exposure through agricultural work. Small occupational effects were observed on two out of seven tests but may have been the result of multiple comparisons. The authors suggest the failure to find significant association between exposure and neurobehavioural performance may have been a result of exposure misclassification or the fact that workers with poor neurobehavioural performance may have quit their jobs and not been included in the study. Inclusion of individuals with a history of acute exposure and/or alcohol abuse, illiteracy and non-numeracy plus cross cultural issues make the findings of this study very difficult to interpret.

3.3.5 *Greenhouse workers*

Developed countries

Roldan-Tapia et al (2005) conducted a cross sectional survey comparing 40 Spanish pesticide applicators with 26 non-exposed controls comprising waiters, cooks, security guards, factory workers and maintenance technicians, matched to the exposed cohort for age and education. Pesticide applicators had been employed for an average of 10 years. None had a history of acute poisoning. Data were collected at a time of high exposure but serum cholinesterase levels were not significantly different between exposed and non-exposed subjects. A relationship was observed between cumulative exposure and delayed verbal memory, visual memory and anxiety levels. Subjects who had been exposed to pesticides for more than 10 years obtained lower scores on tests of integrative perception and visuo-constructional praxis. The authors conclude that long-term exposure to pesticides can cause neurobehavioural problems.

Roldan-Tapia et al (2006) examined the effects of different degrees of pesticide exposure on neuropsychological performance. Data from 24 acutely poisoned workers and 40 non-poisoned but chronically exposed Spanish greenhouse sprayers were compared to 26 controls. Groups were matched for education but the low exposure group was significantly younger than the other two groups. The pesticides used included OPs and carbamates. Chronically exposed subjects were split into two subgroups, high exposure (more than 10 years handling pesticides) and low exposure (less than 10 years handling pesticides). Acutely poisoned subjects had been poisoned in the last 3 months and required treatment in the local hospital at the time of poisoning. Neuropsychological assessment found both the acutely poisoned and highly exposed cohorts obtained significantly lower scores on tests of perceptual ability and visuo-motor processing. Acutely poisoned individuals also showed evidence of verbal and perceptive memory deficits and impaired constructive abilities. Subjects with high chronic exposure and acutely poisoned individuals had similar neuropsychological profiles.

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

3.3.6 Studies of UK Farmers

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

The Institute of Occupational Medicine (1999) carried out three phases of research into the

relationship between long-term, low-level exposure to OPs and ill health. The first phase of the study was designed to quantify the uptake of OPs in relation to procedural and behavioural aspects of sheep dipping. The results showed that the most important source of exposure was skin contact with concentrated sheep dip, which almost always occurred when the farmer handled concentrate containers in order to dilute the product and replenish the dipping bath. The second phase was a cross-sectional study of exposure to OPs and symptoms of peripheral neuropathy. The third phase of the study is most relevant to the current review and was reported by **Jamal et al (2001)**. 74 individuals who participated in phase 2 were classified into three groups according to whether they had signs of peripheral neuropathy ('no', 'possible' and 'probable/definite' signs) and their performance on neuropsychological tests was related to these groupings. Those with neuropathy had poorer mental health. Tests of memory, attention and reaction time were administered. No consistent differences between the groups were found on any of these measures. The IOM acknowledged that their sample size was too small to allow a meaningful analysis of the relationship between cognitive function and exposure history. Exposure history was not specified or used as a variable in the analysis. The majority of psychometric tests administered were visual and only one verbal memory test was included despite the fact that previous studies suggest verbal functions may be affected. The study design is unusual in that it assumes there should be a relationship between peripheral nerve damage (neuropathy) and central nervous system damage (cognitive function) but this may not be the case, indeed recent studies suggest that peripheral nerve damage and central nervous system damage can be dissociated and that the mechanism underlying each condition may be different (Abou-Donia, 2005). Overall, the value of phase 3 of this study is limited.

Stephens et al (1995) studied the effect of low-level chronic exposure in 146 Farmers who had been exposed to OP sheep dip for an average of 15 years and compared them with 143

unexposed quarry workers. A period of 2 months was imposed between any use of an OP pesticide and neurobehavioural testing to ensure the absence of acute effects. The farmers performed significantly worse than controls on tests of sustained visual attention, speed of information processing and syntactic reasoning (a finding replicated by Stephens and Sreenivasan, 2004). They did not perform worse on tests of memory. They also showed greater vulnerability to psychiatric disorder. The authors concluded that repeated exposure to OPs appears to be associated with subtle changes in the nervous system, but that these are unlikely to be manifest as clinical symptoms. However, the farmers and controls differed in terms of educational level, alcohol consumption, and first language. Stephens et al did not report whether any of their farmers had a history of 'dippers flu', making it impossible to determine whether any participants had a history of acute poisoning. Nevertheless, this study raised concern about the effects of chronic exposure to OPs. Indeed, **Beach et al (1996)** followed up 20 of these farmers and split them into two groups according to how many symptoms they reported after dipping. The 10 most symptomatic and 10 least symptomatic farmers then underwent a neurological examination several months after dipping and were compared to 10 unexposed controls. Although the prevalence of neurological abnormalities was low amongst the farmers, subtle adverse neurological effects were detected involving two point discrimination in the hands and feet and calf circumference. Stephens et al (1996) also investigated whether a relationship exists between acute symptoms suffered immediately after dipping and the development of chronic neurobehavioural problems later. However, they did not find any evidence of an association and they suggest chronic neurobehavioural effects occur independently of acute symptoms of exposure.

Mackenzie Ross et al (2007) examined 25 farm workers with a history of apparent low level exposure to sheep dip and compared them to 22 non-exposed healthy volunteers recruited from job centres and newspaper advertisement. Two thirds of farm workers had

retired or reduced their workload on ill health grounds and all were involved in litigation. They performed significantly worse than non-exposed healthy volunteers on tests of mental flexibility, response speed and memory; and over 70% suffered from mood disorder. Although this study included participants who had retired on ill health grounds, the sample size was small and self selected making it unclear how representative they are of the farming community as a whole. Furthermore, many farm workers appeared to have a history of undiagnosed acute poisoning.

In 2010 Mackenzie Ross et al reported the findings of a much larger study of UK sheep farmers exposed to low levels of OPs. Methodological weaknesses of earlier work were addressed by recruiting a random sample of farm workers, including a sub-sample who had retired on ill health grounds; excluding participants with a history of acute poisoning, medical or psychiatric conditions that might otherwise account for ill health; and exploring factors which may render some individuals more vulnerable to the effects of OPs than others. Performance on tests of cognition and mood of 127 exposed sheep farmers (67 working; 60 retired) was compared to 78 unexposed controls, comprising rural police workers (38 working; 40 retired) matched for age, years in education and premorbid IQ. Farmers performance on psychometric tests was also compared with published test norms derived from a cross section of several thousand adults in the general population. Farmers had been exposed to OPs for an average of 24 years but most had stopped using OP pesticides once compulsory dipping ended in the early 1990s. Over 40% of the exposed cohort reported clinically significant levels of anxiety and depression compared to less than 23% of controls. Examiners acquired information about farmers' exposure histories after completing the neuropsychological assessments. Exposed farmers performed significantly worse than controls and standardisation samples on tests of memory, response speed, fine motor control, mental flexibility and strategy making even after controlling for the effects of mood. The

pattern was similar for both working and retired cohorts. The authors conclude a relationship may exist between low level exposure to OPs and impaired neurobehavioural functioning.

3.4 Summary

Thirteen out of sixteen studies reviewed in this paper found evidence of neurobehavioural impairment following long-term, low level exposure to OPs, ranging from subtle deficits in one or more areas (usually reaction time and fine motor control: Fiedler et al, 1997; London et al, 1997; Steenland et al, 2000; Stephens et al, 2004) to major deficits in several cognitive domains (memory, attention, reaction time and visuo-spatial deficits; Bazylewicz-Walczak et al, 1999; Farahat et al, 2003; Mackenzie Ross et al, 2007; Mackenzie Ross et al 2010; Roldan-Tapia et al 2005 and 2006; Srivastava et al, 2000; Stephens et al, 1995). Emotional difficulties were also frequently reported (Amr et al, 1997; Farahat et al, 2003; Mackenzie Ross et al, 2007; Mackenzie Ross et al 2010; Steenland et al, 2000; Stephens et al, 1995).

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

overall value of these three studies is limited by major methodological weaknesses.

3.4.1 Potentially critical exposure variables

Studies which found subtle neurobehavioural deficits following exposure were of pest control operators (Steenland et al, 2000) and fruit tree farmers (Fiedler et al, 1997; London et al, 1997; Stephens et al, 2004). All studies included adequate outcome measures, although London et al (1997) had to modify their measures because of cross cultural issues. The study of pest control operators by Steenland et al (2000) involved study participants who had a relatively short history of exposure to OPs (average of 2.4 years) and this may account for the minimal findings. Studies by Stephens et al (2004) and Fiedler et al (1997) involved small sample sizes with limited power to detect associations, particularly small effect sizes. The study by London et al (1997) is particularly difficult to interpret due to a number of methodological weaknesses including the inclusion of exposed persons in the control group and persons with a history of acute exposure in the exposed groups. It is possible that the exposure history of fruit tree farmers and pest control operators differs in some important way from other types of agricultural work (e.g. sheep dipping or greenhouse work) or the manufacture of OPs, but more detailed information about the working practices of these different occupational groups would be required to determine if this is the case and could account for the different findings.

Remaining studies indicate that both intensity and/or duration of exposure may be important variables underlying the development of neurobehavioural problems. Studies by Srivastava et al (2000), Amr et al (1997) and Farahat et al (2003) of chemical plant manufacturers and Egyptian pesticide formulators and applicators describe their study participants as having fairly prolonged, continuous, daily exposure to OPs as opposed to brief seasonal exposures reported in some other occupational groups such as sheep dippers. For example, formulators

work 40 hour days, every day and Egyptian applicators work 120 days per year. This contrasts with sheep dippers who may only be exposed to OPs on four occasions a year. Srivastava et al (2000), Amr et al (1997) and Farahat et al (2003) all found evidence of significant neurobehavioural problems following long-term exposure to OPs. Studies by Roldan Tapia et al (2005 and 2006) and Bazylewicz-Walczak et al (1999) of greenhouse workers found an association between cumulative exposure and neurobehavioural problems, particularly in those exposed for more than 10 years. The importance of ‘prolonged exposure’ was echoed by Mackenzie Ross et al (2007) who found an association between duration of exposure and impaired memory and motor function in a group of sheep dippers with an average of 14 years of exposure to OPs. All of these studies suggest neurobehavioural problems develop over several years and not after a single episode or season of exposure and that intensity and/or duration of exposure are critical causal factors.

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

3.4.2 *How robust is this synthesis?*

Clearly the individual studies described in this narrative review differ in terms of methodological quality and study populations and these factors may explain the variability in study findings. Although the majority of studies find an association between long term, low level exposure to OPs and impaired neurobehavioural function it is not clear which results are most reliable and should be used as the basis of policy decisions. It is important to get a clear answer to the question of whether low level exposure is harmful to human health, as many more individuals are likely to be at risk of long-term, low level exposure, rather than acute poisoning.

4. Meta-analysis

Meta-analysis is a useful method of summarising, integrating and quantifying the results of different studies to establish if an association exists between specified variables in a group of studies. It combines information across studies thereby increasing the number of participants, reducing random error, narrowing confidence intervals and increasing statistical power to detect small effects that may be missed by individual studies which are too small to yield a valid conclusion (Zhou et al, 2002; Centre for Reviews and Dissemination (CRD), 2009). It represents each study's findings in the form of effect sizes. Combining the results of several studies in this way gives a more reliable estimate of whether a significant association exists between specified variables, than one study alone. Meta-analysis moves discussion away from individual studies towards an overview of a body of literature and it is considered to be the method of choice in situations where research findings may be used to inform public policy (CRD, 2009). The remainder of this paper reports the findings of a meta-analysis of the literature previously described. As far as the authors are aware, this will be the first systematic review of the literature concerning the neurotoxicity of low level exposure to OPs to attempt quantitative evaluation of study findings using meta-analysis.

4.1 Selection of studies

While the 16 epidemiological studies described in this review were identified as being suitable for inclusion in a meta-analysis, two of the identified studies (Jamal et al 2001; London et al 1997) failed to include sufficient data to calculate effects sizes such as sample sizes, means or standard deviations and had to be excluded. For example, London et al (1997) did not provide means and standard deviations for exposed and control subjects separately, but aggregated the data in their published paper. Jamal et al (2001) classified 74 UK sheep dippers into three groups according to whether they had signs of peripheral neuropathy, however exposure history was not specified or used as a variable in the analysis and data from appropriately matched controls were not provided. The overall study design is quite different from that involved in the other studies included in the review which undertook group contrasts involving exposed and unexposed populations. Jamal et al's study was therefore not considered comparable to the others included in the meta-analysis and was excluded from this review.

Three other studies either failed to report means and standard deviations for all of the group contrasts undertaken (Ames et al 1995; Rodnitzky et al 1975; Steenland et al) and merely stated their findings were non significant, in which case an effect size of zero was assigned rather than omitting the study altogether, since this might have biased the results. However, it is important to note that this procedure leads to effect size estimates that are small and is very conservative in nature (Rosenthal, 1995).

Finally, the study by Amr et al 1997 was included in only one part of the meta-analysis as it had a limited focus which was to determine the incidence of psychiatric disorder in pesticide applicators and formulators. Assessment of cognitive functioning was not undertaken.

Table 2 summarises the 14 studies included in the meta-analysis. The aim of all of these

studies was to determine the effect of long term, low level exposure to OPs on neurobehavioural function, but researchers examined a broad range of populations from chemical plant workers, pest control operatives, greenhouse workers, crop sprayers, sheep dippers and fruit tree sprayers. Studies were carried out on individuals from developed and developing nations and exposure history varied considerably from being continuous i.e. on a daily basis, to seasonal or infrequent (e.g. twice a year). Lifetime exposure also varied from an average of two to over twenty years.

INSERT TABLE 2 HERE

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

4.2 Calculation of effect sizes and effect size formulas

Many measures of effect size have been proposed and the most common are Pearson's correlation coefficient, r , Cohen's d (and its multiple variants such as Hedges' g , Glass's Delta etc), and the odds ratio (OR) (Field & Gillett, 2010). Since all of the papers selected for meta-analysis involve group contrasts, Cohen's d seems the most appropriate formula for the current meta-analysis as it is based on the standardised difference between two means. It is calculated by subtracting the mean of one group from the mean of another and standardising it by dividing by the population standard deviation. However, previous research suggests that exposure to OPs may have differential effects on different individuals and therefore exposure will not only affect the mean of any outcome variables used in a study but also the variance. In such cases it is best to estimate the effect size using only the standard deviation of the control group because it is a better estimate of the population variance (Lipsey & Wilson, 2001). Glass's Delta is a variation of Cohen's d which uses the standard deviation for the control group when calculating effect sizes:

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

The meta- analysis was performed in several stages. Firstly (step 1), multiple effect sizes were calculated for each study incorporating data from all of the psychometric tests

administered in a given study, but omitting the data from mood questionnaires as the latter is based on subjective self report rather than objective measures of cognition. However, results could be biased by a small number of studies producing multiple effect sizes, so an overall effect size was calculated per study so that each study contributed a single effect size. Thus before undertaking the meta-analysis across studies a single mean effect size within each study was computed by adding up the effect sizes for each variable of comparison and then dividing this number by the number of comparisons made. The second stage of analysis (step 2) involved examination of effect sizes found in different studies and establishing the variance of effect size distributions (heterogeneity) to determine whether studies are comparable. Finally the influence of potential moderator variables on the overall findings was considered such as task parameters (outcome measures) and population characteristics (of both exposed and control samples).

4.3 Method of meta-analysis

All analyses were conducted using custom-written syntax for SPSS. The meta-analysis was computed by the Mix 1.7 programme (Bax et al, 2006) and a random effects model was used as it is assumed that there will be random differences between studies which are not solely due to sampling error, but are associated with variations in procedures. Random effects models are generally considered to be more appropriate than fixed effects models when analysing behavioural, social and health science data (Field & Gillett, 2010).

4.3.1 Step 1 – establishing study effect sizes using Glass’s delta.

Table 3 shows the included studies, overall single effect sizes for each study based on the mean. Effect size calculations using Glass’s formula are reported and the number of psychometric tests administered in each study upon which the effect size calculations were based appear in the final column.

INSERT TABLE 3 HERE

4.3.2 Step 2 – analysis of the findings by study.

Various graphical techniques exist to illustrate the central tendency, variability and normality of effect size distributions and the stem and leaf, forest and funnel plots are particularly popular. Figure 1 is a forest plot depicting the effect sizes, 95% confidence intervals and the amount of variation between studies. Note that Amr et al (1997) is not included in this figure due to the reasons cited in Section 4.1 above.

INSERT FIGURE 1 HERE

The first thing to note is the direction of the effect sizes; eleven showed a negative effect, and two showed a positive effect. If no consistent pattern existed then one would expect to see a random pattern of effect sizes scattered in both directions at a 50:50 ratio. A 2-tailed binomial test (with .5 set as the test proportion) revealed that the proportion of negative effects sizes seen in these studies were significantly higher than expected ($p=.04$). This predominantly negative pattern indicates poorer performance in exposed workers than unexposed controls. There were only two exceptions to this. Firstly, Ames et al (1995) failed to report necessary statistical parameters for the majority of the psychometric tests in their study. In these cases, effect sizes of zero were assigned before undertaking the meta-analysis. This is a conservative approach which is likely to have lowered the overall effect size for this study. Secondly, Roldan-Tapia et al (2005) failed to find significant differences in performance between exposed and unexposed populations on the vast majority, but by no means all, of the tests included in their assessment battery. More than twenty tests of neurobehavioural functioning were included in the assessment battery, but exposed and control subjects obtained similar scores on the vast majority of tests. This may be why overall, a negative effect size was not apparent.

Most of the effect sizes illustrated in Figure 1 cluster around -.03 (overall ES -0.3148, $p < 0.0053$) but there is some variation in effect sizes ($\tau^2 = 0.1168$; if τ^2 is near to zero then any dispersion in effect sizes is due to random error. When τ^2 moves away from zero it suggests some of the variance is real and due to fundamental methodological differences between studies) with studies by Srivastava et al (2000) and Mackenzie Ross et al (2007) showing the largest effect sizes.

Srivastava et al (2000) was the only study to examine Indian chemical plant manufacturers but unexposed control subjects were not matched to the exposed group for level of education and would be expected to outperform the chemical plant workers on neurobehavioural tests. This may explain why the effect size produced by this study was larger than that observed in other studies. Having said that, a number of other studies have utilised unmatched control groups (Ames et al, 1995; Fiedler et al, 1997; Steenland et al 2000; Stephens et al, 1995) and the first two of these produced low or zero effect sizes; so an alternative explanation might be that Srivastava et al's study participants had more prolonged exposure than other groups as they were involved in the manufacture of OPs on a daily basis rather than the occasional, seasonal application of OPs.

Mackenzie Ross et al (2007) examined 25 farm workers with a history of apparent low level exposure to sheep dip but the sample size was small and self selected making it unclear how representative they are of the farming community as a whole. Mackenzie Ross et al's sample is different from others reported in the literature in that a large proportion of study participants had retired on ill health grounds, whereas other studies recruited participants who were still fit enough to be in employment. Furthermore, participants in the Mackenzie Ross et al study were involved in litigation and so there are a number of factors such as potential

secondary gain or the possibility that participants constitute a subgroup of people who are particularly vulnerable to the effects of OPs, which could explain the large effect size produced by this study.

In order to determine whether the Mackenzie Ross et al study, which produced the largest effect size, was biasing the findings, analyses were repeated excluding this study (see Table 4). It is possible to statistically test for homogeneity to determine whether effect sizes from different studies show more variation than would be expected from sampling error alone and gives an indication of whether studies are broadly comparable (Lipsey and Wilson, 2001). Random effects meta-analyses provide a measure of absolute variance reported as τ^2 . As mentioned earlier, if it is near to zero then any dispersion in effect sizes is due to random error. When τ^2 moves away from zero it suggests some of the variance is real and due to fundamental methodological differences between studies.

INSERT TABLE 4 HERE

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

4.4 Influence of study publication date

Another interesting observation from the forest plot depicted in Figure 1 is the fact that nine

out of ten studies published after 1995 found negative effect sizes between -0.03 and -1.62 , the only exception being a study by Roldan Tapia et al in 2005 which produced a positive effect size of 0.04 . The earlier studies by Ames et al (1995) and Rodnitzky et al (1975) which produced the lowest effect sizes were beset by methodological weaknesses such as failure to provide adequate information about exposure history and/or to report means and standard deviations for all of the group contrasts undertaken.

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

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

4.6 File drawer analysis

One potential bias in meta-analysis arises from the fact that significant findings are more likely to be published than non significant findings and this is known as publication bias or the ‘file drawer problem’ (Field & Gillett 2010; Rosenthal, 1995). In order to address the specific concern that studies with negative findings may not be published, a statistic known as the fail safe N can be calculated. This estimates the number of unpublished studies reporting null results that would need to exist to turn a significant population effect size estimate into a non-significant one. Lipsey and Wilson (2000) suggest the following formula for the fail safe N:

$$k_o = k(ES_k / ES_c - 1)$$

where k_o is the number of non-significant studies needed to reduce the mean weighted effect size to the criterion effect size, k is the number of studies in the analysis, ES_k is the effect size of the meta-analysis and ES_c is the criterion effect size. As it is not possible to divide by zero, the ES_c in this case was set to 0.01. Using this formula, the number of studies with a zero effect needed to make the results of the current meta-analysis non significant would be 301. Thus publication bias is not a significant concern, and this analysis rather robust.

4.7 Effect of cognitive task

Neuropsychological tests are useful tools for exploring the early effects from exposure to toxic substances (Lezak, 1984; Lucchini et al, 2005) but tests vary in terms of their sensitivity to neurotoxic effects and clinical utility for toxicity diagnoses (Hartman, 1995). Some cognitive functions appear to be affected to a greater degree than others by exposure to OPs and tests of psychomotor speed, reaction time, fine motor control, attention and memory are particularly sensitive to OP exposure. Non verbal abilities tend to be affected to a greater degree than verbal abilities although why this should be the case is poorly understood (Anger

et al, 1996; Anger et al, 1997; Anger et al, 2000; Hartman 1995; Lucchini et al 2005). In contrast tests of vocabulary and general knowledge do not appear sensitive to neurotoxic effects, but are often included in assessment batteries as estimates of premorbid ability.

The current meta-analysis incorporated data from all of the psychometric tests administered in a given study (i.e. multiple effect sizes were calculated) and then a single mean effect size within each study was computed before undertaking the meta-analysis. To determine whether task parameters might influence effect sizes the meta-analysis was repeated but this time cognitive tests were grouped into cognitive domains and a single effect size was calculated for each domain by averaging the effect sizes across all measures within that domain. The process by which neurbehavioural tests were assigned to specific cognitive domains was somewhat arbitrary and is illustrated in Table 5.

INSERT TABLE 5 HERE

Table 6 summarises the results of meta-analysis by cognitive domain.

INSERT TABLE 6 HERE

The neuropsychological tests which produced the largest effect sizes included tests of working memory/attention, visual memory, psychomotor speed, executive function and visuo-spatial ability.

5. Discussion

This literature review was carried out to investigate the functional consequences of long term low level exposure to OPs. Although more than 600 published papers were identified concerning the impact on health of exposure to OPs, the vast majority were excluded as they did not address low level exposure to OPs and neurobehavioural functioning in adult populations. After removing articles that had been duplicated by different search strategies,

failed to meet exclusion and inclusion criteria or failed to provide relevant statistical information required for meta-analysis, a final sample of fourteen studies were identified as suitable for inclusion in this review and meta-analysis. The majority of studies were of individuals who had been exposed to a mixture of pesticides, OPs being just one of the chemicals involved. All studies involved comparisons of exposed and unexposed individuals and provided quantitative measures of exposure and neurobehavioural outcomes.

Meta-analysis was used to assimilate the data from these studies in order to determine the extent and nature of any association between exposure to OPs and cognitive impairment. Meta analysis is only meaningful if the aggregated studies deal with similar constructs / relationships and utilise similar statistical analyses, hence the need for strict inclusion and exclusion criteria to limit the review to comparable (homogeneous) studies.

Data from more than 1,600 participants was aggregated in order to produce a more reliable estimate of the association between exposure to OPs and neuropsychological impairment. The analyses show that overall a significant association exists between exposure to low levels of pesticides containing organophosphates and decrements in cognitive function which is small in magnitude. Working memory/attention, visual memory, psychomotor speed, executive function and visuo-spatial ability were affected to a greater degree than other cognitive domains such as language and general knowledge.

Methodological differences between studies make it difficult to comment further on the precise nature of the relationship between exposure to OPs and neurobehavioural functioning. A number of important questions remain unanswered, for example, the critical exposure variable remains unclear; is it dose, intensity, frequency or duration of exposure? Retrospective studies investigating the impact on human health of repeated exposures to OPs struggle to obtain detailed information regarding dose, frequency and intensity of exposure,

making it impossible to determine dose/response relationships. This is because objective evidence of exposure in the form of biological monitoring or biomarkers of exposure is rare in many occupations. Indeed, biological monitoring is of limited value in studies of long-term health effects as the human body rapidly metabolises and eliminates toxins making biological monitoring useful for assessing recent, but not long-term exposure. Often the most that can be achieved by researchers is an estimate of level of exposure based on an individual's testimony regarding the number of years they have worked with a specific chemical product, how frequently they used it and over what time period. Given the limits of human memory, exposure information given in this way may be unreliable. The inability of researchers to obtain precise information about dose, frequency and intensity of exposure probably explains, in part, the continuing debate regarding the relative contribution these variables make in producing toxic effects.

Dose/response effects are frequently assumed to be linear, yet they can be stepwise or curvilinear (Peterson Myers et al, 2009; Hartman, 1999). Researchers should consider the possibility that clear cut dose-response relationships may not be discernible following low level exposure as objective evidence regarding level of exposure is seldom available. Furthermore, there are more than 50,000 OP compounds in existence of differing chemical compositions and their toxic effects vary widely (Karalliedde et al, 2001). The findings from this review illustrate how occupational groups are exposed to a large number of different OP compounds and dose/response relationships are not discernible; neither is it possible to compare the effects of different OP compounds. To complicate matters further, genetic differences between individuals in their capacity to detoxify and metabolise chemicals may render some individuals more susceptible to the effects of certain chemicals and compound any dose/response relationships which may exist. Dose-response relationships may be mediated by other factors such as the synergistic effects of chemical combinations? The

findings from this review illustrate how occupational groups are frequently exposed to a large number of chemicals, OPs being just one of the substances present in these mixtures.

Other important questions which remain unanswered include: The time course of development of neurobehavioural problems and whether they can be ameliorated? The mechanism underlying neurobehavioural dysfunction and the relationship, if any, to acute cholinergic effects or peripheral nerve damage. Other potential non-cholinergic mechanisms which may underlie neurobehavioural changes have been proposed such as alterations in neurotransmitters such as dopamine, changes in receptor numbers or sensitivity; inhibition of other enzymes and proteins (Jamal, 1997; Pancetti, Olmos, Dagino-Subiabre, Rozas & Morales, 2007; Pope, 1999) and apoptotic neuronal cell death (Abou-Donia, 2005; Kapur, Radotra, Minz & Gill, 2007).

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

5.1 What are the critical exposure variables?

Although the current review utilised strict inclusion and exclusion criteria to limit the analyses to studies incorporating similar methodologies, there remained a degree of heterogeneity amongst studies, most notably in terms of the populations examined. Different

occupational groups were evaluated including chemical plant manufacturers, pest control operatives, greenhouse workers, fruit and crop sprayers and sheep dippers. These populations differ considerably in terms of intensity and frequency of exposure which can range from a couple of days a year to several months or even daily exposure in the case of manufacturers. Indeed, studies which have included urinary analysis of OP metabolites suggest concentrations can vary enormously (e.g. 10-200 fold) between different occupational groups such as US pesticide applicators, orchard tree sprayers, termite control applicators and Egyptian cotton field applicators (Farahat et al, 2010).

The populations included in this review also differ in terms of their country of origin, some of the largest effect sizes being produced by studies from developing nations (Amr et al 1997; Farahat et al 2010; Srivastava et al 2000) where daily exposure is not only more frequent and intense, but workers may not receive pesticide safety training or wear suitable protective clothing (Farahat et al, 2010). Heat and humidity may alter the characteristics and toxicity of chemical products and influence decisions regarding the use of personnel protective clothing. Linguistic differences and possible illiteracy may mean instructions for use, storage and other health and safety advice are not followed and economic factors may mean products that have been banned from other countries due to health and safety concerns may still be in use.

Lifetime cumulative exposure may also be an important variable underlying the development of neurobehavioural problems and this also ranged considerably between studies from as little as 2 years to over twenty years. Srivastava et al (2000), Amr et al (1997) and Farahat et al (2003) all found evidence of significant neurobehavioural problems following long-term exposure to OPs. Studies by Roldan Tapia et al (2005 and 2006) and Bazylewicz-Walczak et al (1999) of greenhouse workers found an association between cumulative exposure and neurobehavioural problems, particularly in those exposed for more than 10 years. The

importance of ‘prolonged exposure’ was echoed by Mackenzie Ross et al (2007 and 2010) who found an association between duration of exposure and impaired memory and response speed in sheep dippers with an more than 14 years of exposure to OPs. All of these studies suggest neurobehavioural problems develop over many years and not after a single episode or season of exposure.

It is important that future researchers group and analyse studies by occupation and country of origin because exposure history varies greatly between different occupational groups and even between nations (Farahat et al, 2010). A variety of factors influence the amount of exposure an individual worker might have including the nature of the work (spraying, dipping, ground application), hours, days, years spent working with pesticides; whether the worker is exposed to a single chemical or a mixture of chemicals, use of protective measures (whether machinery was used to apply the pesticides, whether workers were protected by being in sealed cabs or using respirators or other protective clothing) environmental differences in temperature, humidity etc. It is also important to note that important differences may exist even within occupational groups for example the exposure histories of farm workers/pesticide applicators in different regions of the USA vary considerably (Starks et al, 2012; Alavanja et al, 1996). Some regions employ migrant workers who live in camps adjacent to fields where chemicals have been sprayed. This is not the case in California or Washington State both of which have strict regulations for the protection of farm workers and a surveillance programme for reporting pesticide related illness.

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

Another issue raised by this analysis is the possibility that the human health risks of exposure to OPs may have been underestimated by previous studies, because the majority have recruited individuals who are fit enough to be in employment and have not included

individuals who have left the profession because of disabling disease. The only studies which include participants have retired or reduced their workload on ill health grounds were those by Mackenzie Ross et al (2007) and Mackenzie Ross et al, (2010) and this may partly explain the larger effect sizes produced by these studies. Individuals who have retired on ill health grounds may constitute a sub-group of persons who are particularly vulnerable to the effects of OPs either because of their exposure history or genetic factors which may influence their capacity to detoxify chemicals. It is therefore important that future researchers take account of the 'healthy worker' effect and examine individuals who have retired on ill health grounds in addition to those who are still fit enough to be in employment. Measures of susceptibility or vulnerability to the neurotoxic effects of OPs should also be included in future studies. For example human serum paraoxonase (PON1) hydrolyzes and detoxifies a variety of OPs and previous research suggests PON1 status differs amongst individuals (Richter & Furlong, 1999; Richter et al, 2008; 2009; Roest et al, 2007). PON1 polymorphisms may render some people at greater risk of developing ill health following exposure to OPs than others (Cherry et al, 2002; Mackness et al, 2003) and this should be explored by future researchers. Differences in frequencies of resistant genes among different ethnic groups also need to be considered.

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

It is also possible that the human health risks of exposure been overestimated by previous studies because study participants were unrepresentative or high risk groups were recruited (Mackenzie Ross et al, 2007) or inappropriate or unmatched comparison groups may have been used. Steenland et al (2000), Fiedler et al (1997) and Srivastava et al (2000) utilised comparison groups who were more educated than the exposed cohort and would therefore be expected to obtain higher scores on neuropsychological tests because of pre-existing

differences in premorbid ability. Unless further analyses are undertaken to take account of this issue it is difficult to determine the degree to which exposure predicts performance.

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

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

5.5 Conclusion

In summary, the majority of well designed studies find a significant association between long term, low level exposure to pesticides containing organophosphates and impaired neurobehavioural function, which is consistent, small to moderate in magnitude and concerned primarily with neurobehavioural functions such as working memory/attention, psychomotor speed, executive function and visuo-spatial ability. One potential bias in meta-analysis arises from the fact that significant findings are more likely to be published than non significant findings. This is likely to be less of a problem when it comes to research on pesticides as organophosphate pesticides are the most widely used insecticides in the world and prevent millions of people from starving to death and from disease. Studies which produce negative findings are of great interest and are likely to be published as they imply that continued use of these pesticides is safe. Nevertheless, further analyses were undertaken during this review to explore the issue of publication bias and revealed that the number of unpublished studies reporting null results that would need to exist to make the results of the current meta-analysis non significant would be 301. It is therefore unlikely that the association between exposure to OPs and decrements in neurobehavioural function is entirely due to publication bias.

It seems reasonable to conclude that low-level exposure to pesticides containing organophosphates has subtle and specific effects on the central nervous system, resulting in neurobehavioural problems which may not be apparent to health care professionals unless patients undergo formal evaluation utilizing sensitive neuropsychological tests. However, a number of unresolved issues remain in the literature concerning the precise nature of the relationship between exposure to OPs and neurobehavioural function and the strength of the association (has it been under or over estimated). This should be the focus of future studies. A longitudinal prospective study in which neuropsychological function is assessed before, during and after cessation of exposure to OPs would allow many of the unanswered questions

discussed in this paper to be addressed, particularly if it included biological monitoring to determine dose/response effects and analyses of additional non-cholinergic effects which may underlie the development of neurobehavioural change. Unfortunately, the costs involved in running such a project mean it is unlikely a study of this type will be commissioned.

6. Declaration of Interest

None of the authors have any conflicts of interest to declare. The author's affiliation is as shown on the cover page. The authors have sole responsibility for the writing and content of the paper.

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TABLES

Table 1 Inclusion/exclusion criteria for studies in this review

<i>Inclusion criteria</i>	<i>Exclusion criteria</i>
Studies of pesticide exposure where one or more of the ingredients was an OP	Pesticide formulations which do not include OP compounds
Effects of long-term, low level exposure in the absence of an episode of acute poisoning.	Immediate or long-term health effects following acute poisoning. Studies in which individuals with a history of acute poisoning were identified were included in the review if the acutely poisoned subjects were analysed separately from those with a history of low level exposure.
Repeated or prolonged exposure to OPs	Short-term, acute effects following a single episode of exposure
Observational group studies of human adults comparing exposed individuals with unexposed cohorts (controls)	Animal studies, studies of children studies of human adults which did not include an unexposed control group, single case reports
Neurobehavioural outcome measures	Only used outcome measures which are not neurobehavioural eg carcinogenicity, mortality
Objective measures of cognitive function and reliable/validated measures of emotional state.	Questionnaire measures of cognitive function rather than performance on objective psychometric tests. Unstandardised questionnaire measures of emotional state.
	Non-English language papers

Table 2. Table of studies included in the meta-analysis

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
MANUFACTURERS								
Srivastava et al 2000	Health risks associated with the manufacture of OP	59 Indian chemical plant workers 17 controls	Matched for age, sex. Controls better educated.	OP (quinalphos) & other	EHQ AChE	5.7 yrs (sd 4.4)	Medical exam Digit Span Digit symbol Vigilance task	Similar AChE levels in both groups, but exposed had altered reflexes and neurobehavioural deficits, i.e. lower scores on digit span, digit symbol & vigilance task.
Amr et al 1997	Psychiatric morbidity amongst applicators & formulators	208 formulators 172 applicators 233 controls (mix of urban textile workers and rural residents)	Matched for age, socio-economic status, education.	OP, Organochlorines Carbamates, Pyrethroids	Years of exposure	2 yrs	Psychiatric assessment - GHQ, DSM-III-R	Higher depression amongst PF & PA than controls and those with longer duration of exposure (>20 years). Rates of reactive depression equivalent between groups, but rate of dysthymic not & higher than in general population.

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
PEST CONTROL								
Steenland et al 2000	Chronic neurological effects of OP exposure	191 current & former termiticide applicators 189 controls	Matched for age, sex. Controls better educated.	OP (Chlorpyrifos)	EHQ Urinary metabolites PON1	1.8 years (median 2.4 years; range 0.1-10.3 years)	Nerve conduction Clinical exam NES Battery: Finger tapping Hand-eye co-ord Reaction Time Continuous performance Symbol digit BVRT Pattern comparison Pattern memory Switching attention Digit span Serial digit Associate learning Associate recall Mood scales.	Exposed group reported more problems with memory, emotional state, fatigue and muscle strength but few differences noted on quantitative tests. Exposed were impaired on pegboard turning and some postural sway tests but were not significantly different from controls on other cognitive tests. 8 subjects who were acutely exposed had impaired reaction time and continuous performance.

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

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
FRUIT TREE								
Stephens & Sreenivasan 2004	Effect of long-term, low level exposure to OPs on NB function	37 orchard sprayers 26 pig farmers 31 construction workers	Matched for age & education	OP (chlorpyrifos)	EHQ	14 yrs (range 2-25 years)	NB battery same as 1995 study.	Orchard workers slower on syntactic reasoning than controls but no relationship with exposure index.
Fiedler et al 1997	Effect of long-term, low level exposure to OPs on NB function	27 US Fruit Farmers 42 cranberry & blueberry farmers and hardware store controls	Matched for age. Controls more educated.	OP no further data	EHQ	27 yrs (range 5-61 years)	Medical Exam Reaction time Stroop Pegboard Eye/hand co-ord Trails Digit span Digit symbol CVLT Visual reproduction Continuous visual memory test Information Naming Token Test MMPI-2	Exposed and controls had different reading scores and levels of education, so reading score was used as a covariate in the analyses. Fruit farmers have slower simple RT than controls. Fruit farmers split into high vs low exposure and groups differ in simple RT. No other differences found or alterations in mood/personality

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
GREENHOUSE WORKERS (GHW)								
Roldan-Tapia et al 2005	Continuous exposure to OPs (subsymtomatic) and NB effects	40 Spanish GHW 26 matched controls	Matched for age & education.	OP & Carbamates	BuChE EHQ	11 yrs (range 6 months – 30 years)	Medical exam WHO core battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	Association between cumulative exposure and lower performance on verbal memory, visual memory and increased anxiety. Those exposed for more than 10 years also have lower scores on tests of visuo-spatial ability.
Roldan-Tapia et al 2006	Association between different levels of exposure to OPs & NB function	24 Spanish GHW with a history of acute exposure. 40 workers with low level exposure (high vs low groups) 26 controls	Matched for age & education, but low level exposure group younger than other two groups.	OP (metamidophos, fenamiphos, malathion, fosetyl, dimethoate) & Carbamates	BuChE EHQ	11 yrs (range 6 months – 30 years)	Medical exam WHO core battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	Subjects had reduced visuo-motor, perceptual & constructive abilities, verbal learning, speed of processing and increased anxiety. Acutely exposed and those exposed for > 10yrs had similar profile of deficits. Those exposed for <10yrs and controls had similar profiles.

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
Bazylewicz-Walczak et al 1999	Behavioural effects of chronic exposure to OPs	51 Polish GHW (female) 25 controls (admin, canteen workers) matched for age,educ	Matched for age, education, alcohol use & smoking.	OP (dichlorvos, methamidophos, methidathion, pirimiphos-methyl) Carbamates Pyrethroids Dithiocarbamates	Air sampling Concentration on clothes	12 yrs (range 1-24 years)	WHO battery: Reaction time Digit Symbol Digit span BVRT Santa Ana Aiming Mood Symptoms Q	No change in performance on NB tests pre/post season, but differences between controls and exposure groups on both occasions suggesting lifetime cumulative exposure affects NB function, but not a single episode of exposure.

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

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

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
Farahat et al 2005	NB effects of pesticide exposure	52 Egyptian PA 50 controls (admin clerks)	Matched for age, sex, education	OP (chlorpyrifos, Dusban, Curacran, Hostathion, Thimet, Profenofos, Triaziphos, Phorate), Carbamates, Pyrethroids	AChE EHQ	18 yrs (sd 8.29)	Medical Exam Similarities Digit symbol Trails Block Design PASAT Letter cancel Digit span Benton visual form discrimination test Story recall EPQ	PAs obtained lower scores on similarities, tests of attention, visual memory and timed tests than controls and this did not correlate with AChE levels (so not due to current exposure) but did correlate with lifetime exposure. Neuroticism higher and symptoms of numbness & dizziness.

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

Study	Research question	Participants	Controls matched	Pesticides	Exposure Measures	Average exposure	Measures	Results
SHEEP DIPPERS								
Stephens et al 1995	Repeated, long term exposure to OPs & NB function	146 sheep farmers 143 controls (quarry workers)	Farmers older and more educated. Controls consume more alcohol.	OP (diazinon, chlorfenvinphos, propetamphos)	EHQ	15 yrs (range 2-45 years)	Digit span Visual memory Reaction time Digit symbol Syntactic reasoning Word learning Category search	Farmers slower than controls on all timed tests, impaired attention but memory intact. Split into 5 levels of exposure groups and highest exposure group worst on syntactic reasoning (even after controlling for covariates) Greater vulnerability to psychiatric disorder.
Mackenzie Ross et al 2007	Nature & extent of NB problems in farmers who report chronic ill health.	25 sheep dippers 22 controls	Matched for age, sex, years in education.	OP (diazinon, chlorfenvinphos, propetamphos)	EHQ	14 yrs (range 3-32 years)	WAIS-R AMIPB Trails A&B Face recognition Line orientation verbal fluency NART Stroop HAD (mood)	Exposed had lower scores on tests of mental flexibility, verbal memory and 70% had mood disorder. Many reported 'dippers flu' which may be indicative of unrecognised acute toxicity.

Mackenzie Ross et al 2010	Repeated, long term exposure to OPs & NB function	127 sheep dippers 78 controls	Matched for sex, years in education.	OP (diazinon, chlorfenvinphos, propetamphos)	EHQ	24 yrs (range 5-66 years)	WAIS-III WMS-III Trails A&B Graded naming WTAR Verbal fluency Grooved Pegboard Stroop CALCAP Symptom validity test HAD (mood) BDI-2 BAI SCID	Exposed subjects had a higher incidence of clinically significant depression and anxiety than controls. They also performed significantly worse than controls on tests of memory, response speed, fine motor control, mental flexibility and strategy making, even after controlling for the effects of mood.
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OP organophosphate; NB neurobehavioural; PA pesticide applicator; PF pesticide formulator; AChE acetylcholinesterase; BuChE serum cholinesterase; PON1 paraoxonase 1; EHQ exposure history questionnaire; GHQ general health questionnaire; DSM-III diagnostic and statistical manual 3rd edition; AMIPB adult memory and information processing battery; HAD hospital anxiety and depression scale; BDI-2 & BAI Beck Depression and Anxiety Inventories; SCID structured clinical interview; WHO world health organisation NB core test battery; NES NB evaluation system battery.

Table 3 Effect size calculations using Glass Delta formula, based on population means.

Author	Study date	Participants	Sample size; Exposed vs controls	Glass Delta Mean	Standard error	No of ES per study
Rodnitzky	1975	US farmers And PA	46 23/23	-0.00437	0.30	15
Ames	1995	US PA	135 45/90	0.0515	0.18	7
Stephens	1995	UK sheep dippers	289 146/143	-0.135	0.12	13
Fiedler	1997	US Fruit Tree Sprayers	99 57/42	-0.0975	0.20	18
Bazylewicz-Walczak	1999	Polish GH workers	51 26/25	-0.282	0.28	22
Srivastava	2000	Indian manufacturers	76 59/17	-1.0119	0.28	3
Steenland	2000	Termiticide PA	380 191/189	-0.0327	0.10	11
Farahat	2003	Egyptian PA	102 52/50	-0.541	0.20	12
Stephens	2004	UK FT sprayers	68 37/31	-0.0538	0.24	11
Roldan-Tapia	2005	Spanish GH workers	66 40/26	0.0435	0.25	21
Roldan-Tapia	2006	Spanish GH workers	46 20/26	-0.0296	0.30	42
Mackenzie Ross	2007	UK sheep dippers	47 25/22	-1.617	0.29	21
Mackenzie Ross	2010	UK sheep dippers	205 127/78	-0.652	0.15	23

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

Note: Amr et al (1997) does not appear above for the reasons cited in Section 4.1 of this paper

Table 4. Meta-analysis using a random effects model illustrating the effect of excluding the study by Mackenzie Ross et al.

	Glass Delta Mean <i>Mackenzie Ross et al (2007) excluded</i>	Glass Delta Mean <i>Mackenzie Ross et al (2007) included</i>
Overall ES	-0.2251	-0.3148
95% CI lower	-0.402	-0.5361
95% CI upper	-0.0482	-0.0934
z	2.4939*	2.7867**
τ^2	0.0541	0.1168

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

Note: Amer et al (1997) not included

Table 5. Neurobehavioural tests used by previous researchers and the cognitive domains they were assigned to for the purpose of meta-analysis.

Cognitive domain	Tests	Cognitive domain	Tests
Psychomotor speed	Digit symbol Trails A Reaction Time simple AMIPB Speed Hand/eye co-ordination Tapping	Working memory & attention	Digit Span Tests
Attention & vigilance	Vigilance Continuous performance Letter cancellation AMIPB Task A Sustained attention Paced Auditory Serial Addition Test	Visuo-spatial	Block design Line orientation Benton Visual Form Test WAIS PIQ
Verbal – hold tests	Vocabulary Reading Naming Token Test Sentence WAIS VIQ	Visual memory	Benton Visual Retention Test Pattern memory Face recognition Picture completion Rey Osterieith [ROC] AMIPB figure recall AMIPB design learning Figure Recall Location recognition
Executive function	Similarities Trails B Syntactic reasoning Verbal fluency Reaction time – choice Stroop	Verbal memory	Story recall or Logical Memory Auditory Verbal Learning Test California Verbal Learning Test List learning Serial digit Category learning
		Mood	Anxiety measures Depression measures
		Fine motor control	Santa Ana Test Pursuit Aiming Grooved Pegboard

Table 6. Meta- analyses by cognitive

Cognitive Domain	No studies	Overall ES	Lower CI	Upper CI	z	τ^2
Working Memory	12	-0.338	-0.595	-0.08	2.568*	0.156
Visual Memory	10	-0.0297	-0.532	-0.062	2.475*	0.096
Verbal Memory	9	-0.152	-0.486	0.182	0.893	0.214
Attention	9	-0.263	-0.511	-0.014	2.078*	0.099
Speed	13	-0.531	-0.899	-0.163	2.825**	0.407
Executive function	10	-0.399	-0.796	-0.002	1.969*	0.361
Visuo-spatial	5	-0.37	-0.616	-0.123	2.938**	0.029
Language	7	-0.267	-0.548	0.014	1.864	0.093
FMC	4	-0.462	-1.075	0.15	1.48	0.354
Mood	5	-0.517	-1.044	0.012	1.92	0.31

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

Note: Amr et al (1997) appears above in analyses concerning mood.

ILLUSTRATIONS

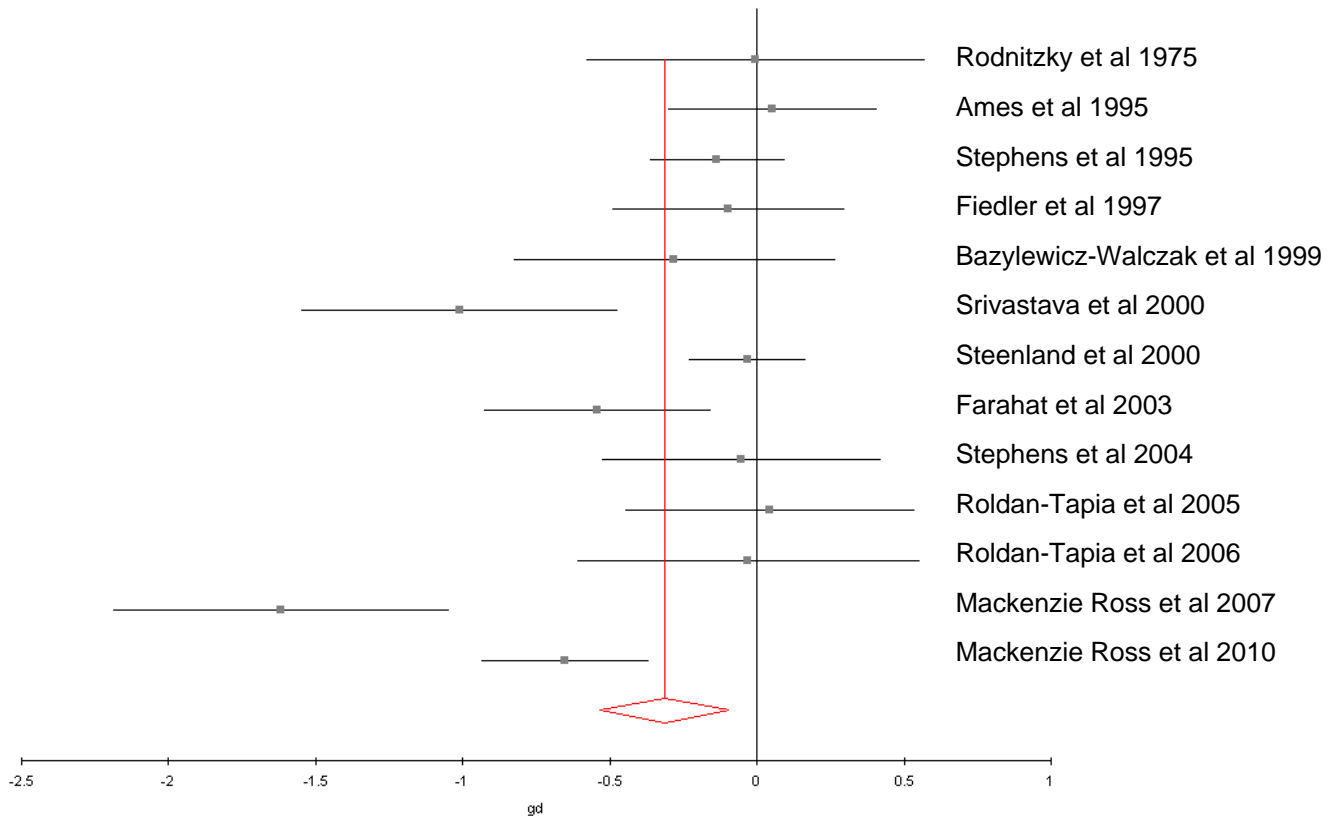


Figure 1. Forest plot depicting effect sizes for each of the studies in date order and 95% confidence intervals.