A systematic review of the influence of occupational organophosphate pesticides exposure on neurologic impairment

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Authors:

Noriko Furuoya[1]

Masahiro Hashizume[1]

Authors' Institutions:

[1] Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki City, Nagasaki, 852-8523, Japan

Address for correspondence:

Noriko Furuoya

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

1-12-4 Sakamoto, Nagasaki City, Nagasaki 852-8523, Japan

Tel: (81) 95 819 7764 Fax: (81) 95 819 7844

E-mail: furuoyanoriko@gmail.com

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ABSTRACT

Background: Organophosphate pesticides (OPs) are widely used: however only a few epidemiological studies have investigated the association between neurobehavioral or neuropsychological effects and occupational OP exposure.

Objective: The aim was to conduct a systematic review of the published literatures and to estimate whether or not there is a causal relationship between occupational exposure to OPs and either neurologic impairment or depressive symptoms.

Method: An extensive search of various literature databases was conducted, and the relevant publications were then manually searched. All the relevant data was extracted from the selected articles and synthesized for analysis. Meta-analysis was implemented using mean scores of the neurologic tests and depressive symptoms.

Results: Twenty-three studies that met inclusion and exclusion criteria were selected for analysis. Of the selected studies, 16 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (nine studies), UK (four studies), Africa (four studies), Asia (three studies), Europe (two studies) and one in South America. The Each study used different exposure and outcome assessments such as neurologic scores and depressive symptoms, thus making it difficult to compare the results exactly. The most showed that the exposed groups had poorer results than the unexposed groups; however, the evidence based on the results of the meta-analysis was weak.

Conclusion: The findings of this literature review indicate that there might be a causal relationship between occupational exposure to OPs and neurological impairment or depressive symptoms.

ARTICLE SUMMARY

Article Focus

- To systematically review epidemiological studies which examine adverse effects on human central nervous system by organophosphate pesticides (OPs).

Key messages

- OPs have been widely used all over the world for agricultural or industrial use.
- There are a plenty of studies which have examined acute health problems by OPs, however, few studies have investigated negative effects by occupational OPs exposure.

Strengths and limitations of this study

- The article represents a systematic review of epidemiological studies on adverse effects on human central nervous system by occupational OPs exposure, with quality appraisal of each study.
- The meta-analysis was limited because each study used various outcome assessments.
- There is a difficulty to judge negative effects by only OPs, because mixed pesticides were used in some studies.

INTRODUCTION

Ever since organophosphate pesticides (OPs) were developed, they have been used for combating insects for public health purposes and to support agricultural productivity and manufacturing processes. Pesticides are also well-known as one of the leading suicide methods, and approximately three million cases of pesticide poisoning occur every year around the world. This is especially prevalent in Asian nations including Sri Lanka, China, and Malaysia (1). For this reason, a large number of epidemiological studies have investigated the relationship between high level OP exposure such as pesticide poisoning and accidents and acute health effects and it has been reported that high level exposure is significantly related to neurological or neuropsychological impairment (2, 3). In contrast, few studies that have investigated associations between occupational or cumulative OPs exposure and negative effects on human health are available. Although some research has examined the negative influence to young children by cumulative OPs exposure (4, 5) or others have investigated relationships between reproductive health and occupational OPs exposure(6-8), However, there are very few studies which have assessed the relationships between occupational OPs exposure and neurologic or mental problems using epidemiological research. In this systematic review, the epidemiological evidence for the relationship between occupational OPs exposure and mental and neuropsychological aggression is summarized, and some of the limitations associated with the studies discussed.

Materials and Methods

Searching strategy for identification of published studies

A search for observational studies was carried out. Geographical and time restrictions were not imposed. Population-based case-control studies were excluded from the systematic review because it is difficult to assess accurate exposure-doses. Currently, various pesticides including OPs are easily-available for everyone, and some people have a possibility of using pesticides for personal use. However, it is almost impossible to comprehend exactly past records of pesticides use every person. The search was limited to studies in humans and to reports published in English, and the review was limited to epidemiological studies. Studies investigating OP exposure through food and water contamination were also excluded. A search of the following four databases was carried out:

1. EMBASE Classic plus EMBASE (1947 to 2010 July 09),
2. Ovid MEDLINE(R) (1950 to June Week 5 2010),
3. Global Health (1910 to June 2010), and
4. PsycINFO (1806 to July Week 1 2010).

A combination of free-text terms and explore terms was used to identify relevant articles. For exposure, the following search keywords were used: organophosphate*, organophosphorous, pesticide*, or insecticide*, organophosphate pesticide (explore map term), pesticide (explore map term). For outcome, the following search keywords were used: neuro*, psychiatr*, psycholog*, mental health, mental illness, mental disorder, depressi*, depression (Epidemiology) (explore map

term) and mental health (explore map term). For subjects, the following search keywords were used: occupation*, agricultu*, or farm*. For study design, the following search keywords were used: epidemiolog*, cohort, or cross-sectional, case-control, or Epidemiology (explore map term) were used as keywords. An initial systematic search in the titles and abstracts was conducted using a combination of all these search terms. A second manual search of the reference lists from the selected relevant articles was performed to explore or retrieve articles found in the initial search.

Criteria for selecting studies for the review

Only original research articles meeting the inclusion and exclusion criteria described below were used in the final result.

Inclusion criteria

1. Study design:

a) Must be observational studies: cross-sectional, cohort, and case-control studies.

b) Studies must have both exposed and unexposed groups.

2. Subjects:

a) The subjects in the exposed group either must use OPs occupationally, or there must be a probability of being exposed to OPs during their work.

b) The families of occupational OP users can be treated as subjects.

3. Exposure

a) Subjects must be exposed to OPs for at least one month.

b) Seasonal workers who used OPs for more than one month must be included.

4. Outcome

Studies must have carried out some tests to assess damage of the CNS (Central Nervous System) or have conducted a survey or an interview to identify depressive symptoms.

5. Exposure-outcome association

Results must be reported as some type of relative risks or mean scores.

Exclusion criteria

1. Study design

Experimental and laboratory based studies including animal studies were excluded. Population-based case-control studies were excluded.

2. Subjects

Studies of mainly patients of pesticide poisoning were not excluded.

3. Exposure

Studies which did not specify the type of pesticides were excluded.

4. Outcome

Studies examining damage of the peripheral nervous system due to OPs exposure were excluded.

5. Language

Studies published in a language other than English were excluded.

Definitions

Definition of cumulative exposure

a) People who use OPs in their jobs for at least one month and have a probability of inhaling ambient OPs and absorbing OPs by spraying and touching.

b) Families of OP users were included as subjects, because they may have been exposed to OPs by washing clothes contaminated by OPs and/or by touching OP users.

Definition of poor mental health

A) Neurological or neuropsychological impairment

a) People who had poorer results in neurological or neuropsychological battery tests than healthy people of the same age.

b) People who had short-memory loss, for example, people who had experienced memory loss of six to three months duration.

B) Depressive Symptom

c) People who, regardless of their age, had chronic depressive symptoms including headache, fatigue, dizziness, sleepless and eye problems.

d) People who were diagnosed with depression by clinical doctors.

Study selection process

Using the search terms listed above, a total of 592 references were obtained: 276 from Embase Classic + Embase, 16 from PsyINFO, 133 from Global Health, and 167 from Medline. However 197 of 592 references were duplicates. Of these 395 unique references that remained, 63 were not in English, and 32 were animal studies. A manual search of the titles and abstracts of the remaining 300 references excluded a further 268 studies. The 32 remaining articles were fully reviewed, after which 13 studies were deemed to meet inclusion and exclusion criteria (9-21). In addition, 10 articles identified by manual search were added to the systematic review (See Appendix A for flow of study inclusion and exclusion diagram). Finally, these 23 studies were identified and used for data extraction (22-31).

Data extraction, synthesis and analysis

Data extraction forms were created to compare relevant data collected from each of the 23 studies. Extracted data included title, authors, year published, and the number of subjects in the exposed and unexposed groups, occupation, demographic information such as mean age, sex, smoking status, geographical area, inclusion and exclusion criteria such as first language, alcohol consumption, and injury experience, types of pesticides, exposure assessment, statistical methods, outcome assessment to measure the neurologic or neuropsychological ability, and results obtained. Tables containing the

data that was obtained using the data extraction forms were constructed and analyzed. P-values and 95 percent confidence intervals were elicited from the articles to judge statistical uncertainty. When a study had investigated depressive symptoms, the information was collected and a table was constructed. Meta-analysis was carried out using mean scores of neuropsychological tests with STATA version 11.0.

Quality appraisal

The quality of the 23 studies was appraised using a scale that was adapted from the 'Newcastle/Ottawa Scale (NOS)' (32) (The appraisal standard of NOS was shown in Appendix B). Based on the NOS, each study was evaluated using the point system. When a study included relevant information that could be associated to the NOS, one point was added. There are five items in cross-sectional studies and eight items in cohort and case control studies that can be related to the NOS. Therefore, cross-sectional studies assigned 5, 4, 3 or 0-2 points were evaluated as very good, good, satisfactory or unsatisfactory studies, respectively. Similarly, cohort/case-control studies with 7-8, 5-6, 4 and 0-3 points were identified as very good, good, satisfactory or unsatisfactory, respectively.

RESUTLS

As a result of the search strategy described in the Materials and Methods section, 13 studies were

identified from the database search and another 10 studies found after a manual search. A total of 23 articles, published between 1975 and 2010, met the inclusion and exclusion criteria. A summary of the characteristics of the 23 selected articles is shown in Table 1.

Study design and geographical area

Of the selected studies, 16 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (nine studies), UK (four studies), Africa (four studies; two in South Africa, one in Egypt, and one in Kenya), Asia (three studies; two in India, and one in Sri Lanka), Europe (one in Spain and one in Poland) and one in South America (Ecuador, one study).

Characteristics of subjects

Because the subjects were limited to people who had the probability of being exposed by OPs, the majority of the participants were men. Most of the time, agricultural work such as pesticide application and farming is predominantly performed by men. Five out of the 23 studies included both male and female subjects; however, approximately 60 to 70 percent of the subjects were male (9, 21, 27, 29, 33). Only one study used all female subjects in both the exposed and control groups (23). The mean age of the exposed subjects was in the thirties in 12 studies, in six studies the mean age was in the forties (9, 12, 16, 17, 24, 34) and in two studies the mean age was in the fifties (13, 21). The

mean age in one study was in the twenties; however, the mean age was 29, very close to thirty (27). One study did not report detailed demographic data of the participants (10).

Source of recruitment and sample size

Ten out of the 23 studies were on pesticide applicators including private, commercial, and tree, fruit, and vegetable applicators. Four and three studies were on farmers and sheep farmers, respectively, and, two were on factory workers and greenhouse workers. One study investigated depressive symptoms in the spouses of OPs users. In the study by Korsak et al. the specific occupation of the population in the study was not stated; however, the subjects had experienced occupational OPs exposure (25). The number of subjects in the exposed group varied from 16 to 2,051, while the control groups had a wider range, with the figure ranging from 16 to 27,023.

Table1 Reported findings in recent epidemiological studies regarding occupational low level OPs exposure and mental illness

	Author	Study Design	Country	Exposed Population(No)	Chemical	Exposed Assessment	Comparison Group
1	Albers et al (9)	CO	USA	Chemical workers(53)	OP	Industrial HR,AChE INH	Similar workers, not exposed(60)
2	Bazylewicz-Walczak et al (23)	CO	Poland	Greenhouse workers(26)	OP	DR	Greenhouse workers, not exposed(25)
3	Beseler et al (10)*	NC/ CO	USA	Case**: Spouses of private applicators with depressive diagnoses(2,051)	OP	QU or IN	Control: Spouses of private applicators without depressive diagnoses (27,023)
4	Cole et al (33)	CR	Ecuador	Farmers, some applicators(144)	OP,CAR, FNG	IN, QU, AChE INH	Local Population(72)
5	Daniell et al (20)	CO	USA	Farm worker applicators(49)	OP	QU, AChE INH	Slaughterhouse workers(40)
6	Dassanayake et al (13)	CR	Sri Lanka	Vegetable farm workers (38)	OP	N.A.	hospital labours(35)
7	Farahat et al (24)	CR	Egypt	Farm workers(52)	OP	AChE INH	Local Population(50)
8	Fiedler et al (34)	CR	USA	Tree fruit farmers (57)	OP	QU, lifetime exposure metric	Cranbury/blueberry growers(low exposed), hardware storeowners(unexposed) (42)
9	Korsak et al (25)	CR	USA	Occupational exposure(16)	OP, CAR, OC	AChE INH	Local Population(low exposure)(16)
10	Levin et al (26)*	CR	USA	Pesticide applicators(24)	OP	IN, AChE INH	Farmers(24)
11	London et al (18)	CR	South Africa	Fruit farm pesticide applicators(163)	OP	QU(job-matrix)	Farm workers, not applicators(84)
12	London et al(15)*	CR	South Africa	Fruit farm pesticide applicators(164)	OP	QU (job-matrix)	Farm workers, not applicators(83)
13	Maizish et al (27)	CR	USA	Pesticide applicators(46)	OP	UM, DR	Non-applicators(56)

14	Misra et al 5(28)*	PR	India	Pesticide applicators(22)	OP	AChE INH	Hospital labours(20)
15	Ohayo-Mitoko et al (29)*	CO	Kenya	Farm worker applicators(256)	OP, CAR	AChE INH	Farm workers(low exposure)(152)
16	Rodnitzky et al (30)	CR	USA	Pesticide applicators(23)	OP	AChE INH	Farmers(23)
17	Roldan-Tapia et al (14)	CR	Spain	Greenhouse workers(40)	OP, CAR	QU, AChE INH	Local Population(26)
18	Ross et al (21)	CO	UK	Sheep farmers(127)	OP	IN	Police workers(78)
19	Srivastava et al(31)	CR	India	Manufacture workers(59)	OP	AChE INH	Manufacture workers, not exposed(17)
20	Steenland et al(11)	CR	USA	Termiticide applicators(191)	OP	IN,UM	Friends, blue collar workers(189)
21	Stephens et al (12)	CR	UK	Sheep farmers(146)	OP	QU	Quarry workers(143)
22	Stephens et al (17)	CR	UK	Sheep farmers(77)	OP	QU, UM	Quarry workers(69)
23	Stephens et al (16)	CR	UK	Orchard applicators(37)	OP	IN,QU	Construction workers,pig farmers(57)

Study Design CR: Cross-sectional, CO: Cohort, NC: Nested Case-control, PR: Prospective study

Chemical OP: Organophosphates, OC: Organochlorines, CAR: Carbamates, FUN: Fungicides, AChE: Acetylcholinesterase

Exposed Assessment: AChE INH: AChE inhibition, DR: Dermal and Respiratory Absorption, IN: Interview , QU: Questionnaire , HR: Hygiene Records UM: Urinary metabolites,

*Articles including depressive symptoms for outcome assessments

**Cases were defined as female spouses of private applicators who responded 'yes' to the question 'Has a DOCTOR ever told you that you had been diagnosed with depression requiring medication? Controls were female spouses who responded 'no'. (10)

Exposure assessment

Exposure assessment in the included studies was divided, for the most part, into five patterns: indirect assessment using, for example, an interview or questionnaire; direct assessment including the measurement of urinary metabolites and acetylcholinesterase (AChE) levels in the blood or measurement of ambient OPs using a patch and a pump; a combination of direct and indirect methods; and a combination of biomarkers and ambient OP levels. Seven out of the 23 studies used indirect methods, and six studies used blood AChE inhibition levels to measure AChE levels in the blood as an exposure indicator. Six studies used a combination of indirect methods and biomarkers, and three studies used biomarkers and the ambient OP levels. The remaining study did not mention any exposure assessment method. In all the studies which used urinary metabolites as exposure assessment, results were presented as the sum of dialkylphosphates (DAP) (i.e. the sum of six DAP metabolites: DMP (dimethylphosphate), DMTP (dimethylthiophosphate), DMDTP (dimethyldithiophosphate), DEP (diethylphosphate), DETP (diethylthiophosphate), and DEDTP (diethyldithiophosphate)) (8).

Outcome measurements

Two different outcome measurements were used in the studies; one measured neurological impairment and the other assessed depressive symptoms. Of the 23 studies, 18 used cognitive function tests to investigate negative neurologic influences caused by OP exposure.

Associations between outcome and exposure

Ten of the 18 studies investigating cognitive impairment mentioned that at least one measure outcome showed more impairment in the exposed groups; however, these observations were not significant ($P < 0.05$). Six of the studies reported some significant positive associations of exposure with poor outcome ($P < 0.05$); however, even in these cases, the significant decrements were observed only in some of neurologic tests, mainly in the Digit Span and Santa Ana Dexterity tests. Indeed, there are several versions of these neurologic tests and the significance of the scores often depended on versions of the tests that were used. Five studies used the Neurobehavioral Evaluation System (NES), four studies used the Wechsler Adult Intelligence Scale (WAIS), two studies used the World Health Organization Neurobehavioral Core Test Battery (NCTB), and the remaining four studies used their own scales.

Five studies adopted depressive symptoms as outcome measurements as shown in the Table2, however, symptoms used in the studies were not standardized.

Table 2 The Summary table of depressive symptoms

Reference	Obtained Results	Impact of outcomes
Beseler et al 2006(10)	Depression due to doctor's diagnosis was not significantly related to low (OR 1.09; 95%CI 0.91, 1.31) or high (OR 1.09; 95%CI 0.91, 1.31) cumulative exposure.	-
Levin et al 1976(26)	Anxiety score of the pesticide applicators was significantly higher (P<0.05) than that of the farmers. However, there was no significant difference in measures of depression.	++
London et al 1998(15)	Dizziness, sleepiness, and headache had a significantly higher overall neurological symptom score (P<0.05).	++
Misra et al 1985(28)	Common symptoms were Headache(59%), giddiness(50%), ocular symptoms(27%), and paresthesia(18%) and no neurologic change was seen.	-
Ohayo-Mitoko et al 2000(29)	A significant change in symptom prevalence was found for respiratory (2.48% CI(0.78, 5.38) and central nervous system (2.56% CI(0.99, 6.62), but in terms of skin, systematic, and eye symptoms, there was no statistically significant change.	++

OR=Odds Ratio ++: Statistically significant (p<0.05), -: Not statistically significant

Statistical analysis

Sixteen studies used logistic regression, and the remaining seven used other statistical tests including X2-test and t-test. Only one study adjusted for sex in the logistic regression. Thirteen out of the 23 studies adjusted for age, and 11 adjusted for education in the logistic regression. However, only five studies adjusted for alcohol consumption

before carrying out the analysis. Further, only two studies adjusted for first language.

Methodological quality appraisal

Four out of the 23 studies were of very good quality, 10 were of good quality, and the remaining nine were either satisfactory or unsatisfactory. Most of the bad quality studies either were carried out before 1990 or were performed in some of the less developed countries. In particular, the methods of recruitment of subjects, controlling for confounders, and outcome assessment were not appropriate. For example, in some of the studies all of the participants were volunteers (24, 30) and in another study, the subjects were not representative of the community from which they were recruited (factory workers) (31). In addition, how the outcome was assessed was not described in the unsatisfactory studies, and some of the methods needed to avoid confounders such as stratification and regression were not used. On the other hand, none of the cohort studies were assessed as very good quality because most of them did not have a long enough follow up duration (in five studies, the duration was less than six months) and the selected subjects were not fully representative of the target community. Moreover, the methods of outcome assessment were not described in most of the cohort studies.

Data synthesis and meta-analysis

As shown in Figure 1 and 2, a meta-analysis was carried out using the reported mean scores for the implemented neurobehavioral test; however, because the investigators used different scoring systems, meta-analysis was difficult. The commonly used tests in NCTB, NES, and WAIS were Symbol-Digit and Digit Span Forward and Backward. However, some studies that adopted NES and WAIS to measure neurologic impairment (Table3) implemented only a few subsets in the trials. Among five studies using a Symbol-Digit test, three used NES and WAIS, two used WAIS-R and unknown tests, and one was a Polish NCTB. For the Digit Span test, two studies used NES and WAIS in the forward tests and two WAIS in the backward tests. Because there were only two studies in each Digit Span test, a meta-analysis would not be very useful, and so a meta-analysis for the Digit Span tests was not carried out and only a meta-analysis for NES and WAIS Symbol-Digit tests was performed. In terms of Symbol Digit (NES), slight positive association can be seen (Figure 1), while Figure 2 showed that there was no difference in mean score of Symbol Digit WAIS between the exposed and control groups. Although the three studies apparently used the same scoring systems, one of the score was completely different from the other two studies. For example, the scores in the study of Stephens et al. were 24.22 and 21.01 in the exposed and the control groups,

respectively (17), whereas the scores of Daniell and Stephens were much lower: between 2.23 and 3.55 (16, 17, 20). Similarly, the mean scores reported by Bazylewicz-Walczak et al. were higher, 45.50 and 49.40, while the mean scores reported in the other studies were smaller, 2.28 and 2.23 in the WAIS(27).

Table 3 The summary table of neurologic battery tests

Reference	Types of neurologic tests	Symbol Digit	Digit Span	Santa Ana	Simple Reaction Time	Syntactic Reasoning(s)
Bazylewicz-Walczak et al 1999(23)	Polish NCTB/WAIS (Symbol Degit)	nd	nd	nd	+	nd
Cole et al 1997(33)	NCTB	nm	nm	nm	nd	nd
Daniell et al 1992(20)	NES	-	nd	nd	nd	nd
Farahat et al 2003(24)	Unknown	++	++(f)* ++(b)**	nd	nd	nd
Fiedler et al 1997(34)	WAIS-R	-	-	nd	++	nd
London et al 1997(18)	WAIS-R	nm	nm	++	nm	nd
Maizish et al 1987(27)	WAIS	1/++	nd	nd	nd	nd
Roldan-Tapia et al 2005(14)	WAIS	++ †	++ †	nd	nd	nd
Ross et al 2010(21)	WAIS	nd	++	nd	nd	nd
Srivastava et al 2000(31)	Unknown	++	++	nd	nd	nd
Steenland et al 2000(11)	NES	-	-	nd	-	nd
Stephens et al 1995(12)	Unknown	++	-	nd	++	+
Stephens et al 1996(17)	NES/ACT	nm	nm	nd	nm	nm
Stephens et al 2004(16)	NES/ACT	-	-	nd	-	++ (ACTS)

++: P<0.05, +: 0.05≦P<0.1, －: P>0.1,

The Exposed groups were slower or had poor outcomes than control groups

†: The article did not mention whether obtained results were positive or negative
nd: The subsets of neurological tests were not performed
nm: Although the subsets of neurological tests were performed, P-values were not mentioned in the article.
*(f) Digit Span forward, **(b) Digit Span backward

DISCUSSION

The results showed that there were 23 epidemiological studies which examine the relationship between OPs and CNS by systematically searching. When comparing the selected studies by each item, two main findings were obtained; one is exposure assessment and the other is outcome measurement. With respect to exposure assessment, the matter of measurement was categorized into three: direct, indirect and a combination of both methods. On the other hand, in terms of outcome measurements, there seemed to be two main ways to gauge neurologic impairment.

Exposure assessment

Exposure assessment was not used for group allocation in all the studies, and was implemented to measure how much subjects were exposed and the outcomes of the neurobehavioral tests. Each study used different exposure assessment which made it difficult to accurately compare the studies. In addition, there seemed to be methodological imperfection in both the direct and indirect methods. To illustrate,

interviews and questionnaires were used in the indirect method, though, one study recruited subjects over 60 years old who had been 11 years since their retirement (21). In this study, recall bias could be a problem because the rate of cognitive impairment is likely to have increased as the subjects put on years. This could lead to inaccuracy of exposure assessment. With respect to the direct method, there were several ways to detect OPs. Although some studies used urinary metabolites as an indicator of exposure, DPA is metabolized rapidly and excreted from bodies (7). Therefore, measuring urinary analysis was not a perfect way to assess OPs exposure, on the contrary, it seemed that measuring AChE levels was the most reliable way to assess the amount of OP exposure, because the cholinesterase level becomes normal by being synthesized into a new molecular of AChE, which takes around a week (35). Hence, the amount of OP exposure within one week can be accurately measured by AChE inhibition level in blood, but this cannot be assessed the amount of OPs exposure accumulated in body tissues for a long time. Thus, direct method using the levels of AChE in blood is appropriate for assessing short-term exposure, however, it is not for long-term exposure. On the contrary, indirect methods such as structured interview and questionnaire could be helpful to grasp the past information about OPs use, even though there may be some recall bias. In order to minimize measurement error, it is desired that a combination of

direct and indirect methods should be used.

Outcome assessment

As with exposure assessment, a similar problem can be seen in outcome assessment, for example, five out of the 23 studies adopted depressive symptoms as outcome measurements (Table 2). On the other hand, the remaining 18 studies used neurologic battery tests such as NES and WAIS. Thus the main problem in the outcome measurements is that comparison between the studies could not be done easily, because neurologic battery tests differed by each study. To elaborate, as shown in Table 3, three studies adopted WAIS and four used NES as outcome assessment, and since there were various versions of neurologic battery tests including WAIS and WAIS-R, the content of the tests slightly differ from each study. Furthermore, although some studies mentioned about the relationship between OP exposure and confounding factors such as age and education, they did not perform statistical tests between the exposed and control groups. These things obviously make it difficult to compare the outcomes of neurologic impairment among the studies. In addition, even in the same neurologic battery test, there are a variety of subtests such as Symbol Digit and Digit Span to measure neurologic impairment. The studies selected some subtests in their trials, hence there

were few studies left to precisely compare. As a consequence, although the meta-analysis was carried out using the results of Symbol Digit, it was not enough to determine whether or not there was a statistically significant relationship. Similarly, in terms of depressive symptoms, outcomes were different from each study, for instance, one study had the proportion of headache, while the other used that of dizziness and sleepiness as main outcomes. Thus, neurologic battery tests, at least, should be standardized for further epidemiological research. If not, it could be difficult to gain precise conclusion that cumulative OP exposure can negatively affect human CNS or not.

Study design

Sixteen of the studies were cross-sectional studies and six were cohort studies. Longitudinal studies are more desirable rather than cross-sectional studies for three main reasons: one, in cross-sectional studies, it is difficult to confirm whether or not the disease preceded the exposure; two, the outcome conditions in cross-sectional studies are too short-lasting (36); and three, cross-sectional studies are suitable for investigating at a certain point, but they are not appropriate for mid-term studies. Especially, agricultural work using pesticides is easily influenced by seasonality, and one research

regarding reproductive health by OPs exposure stated that sperm concentration and counts are negatively affected on peak season, spring, rather than winter (6). The results of the neurobehavioral tests may also be affected by seasonality; therefore, cohort studies are ideal to assess the influence of occupational OPs exposure than cross-sectional.

Possible bias

If foreign workers are included in the trials, their first language should be considered as possible bias. Because there is possibility that the non-native subjects cannot fully understand the content and instruction of the tests, which could lead to lower score than that of native speakers. Nowadays, USA and gulf countries have accepted foreign workers from India and South American countries as important work force (20, 37, 38). However, in this systematic review, there were only two studies to mention about first language in the inclusion and exclusion criteria (17, 20). Since first language could influence the outcomes, it should be one of the factors to be considered when selecting subjects. Furthermore, when migrants and foreign labourers are included in the studies, education system is a point that we have to pay attention. Because education system between developed and less developed countries could be largely different. Hence, it is

necessary to be careful when the results between subjects who come from different countries are compared. Additionally, occupations could be a factor of selection bias, because police officer and construction workers have a possibility of experiencing the loss of consciousness due to accidents of their jobs (21).

Possible confounders

Age and social cultural factors are known as common confounding factors, though, not all studies adjusted them in the analysis. These factors could easily influence the results; hence they should be adjusted for further trials. Moreover, since head injury and alcohol consumption have a probability of negatively affecting neurologic battery tests, they should be treated as potential confounders as well. However, the results showed that there was no study to adjust head injury in the logistic regression, on the other hand, there were some studies to adjust alcohol consumption in the analysis (10, 15, 18, 20, 27). Apart from these factors, participants' nutrition status including vitamin deficiency is also relevant to the outcome of neuropsychological tests (15, 18). Thus, all factors that can affect measurements of cognitive function should be adjusted in the analysis.

Limitations

Although all of the studies which were collected in this systematic review were relevant to occupational OP exposure, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides usually are mixed with another type of pesticides to make their effects stronger, and this is the common in agriculture. In this systematic review, four out of 23 studies were not single OPs exposure and they used a combination of OPs, OCs carbamates and fungicide. Therefore, it may be quite difficult to measure the effect of only occupational OP exposure.

Of these studies, 18 assessed neurological or neuropsychological impairment using IQ tests. However, since the authors used the different battery tests such as NCTB, NES, and WAIS, there were only a few common tests including Digit Span and Symbol digit tests across the studies, which made the comparison of the included studies more difficult. Hence, a meta-analysis was applied to the two tests, but it is obvious that studies which can be appraised are limited. In order to completely assess neurological impairment, it is desirable that the same neurobehavioral test battery be used in a large number of studies.

CONCLUSION

Although the suggestive evidence for neurobehavioral test battery is inconsistent, there was slight positive relationship of poor outcome implying that occupational exposure to OPs could be harmful for the CNS of the human. The evidence was weak in particular because some studies showed that there was a negative relationship of OPs with poor outcome. In addition, since the test items of the neurobehavioral test battery depended on the investigators, only a few items were common across the studies. Consequently, there were only a few studies left for the meta-analysis; indeed, there were a few items which could be compared. For future studies, the neurobehavioral or neuropsychological test battery should be standardised in order to ensure adequate quality and to make more possible pooling evidence from the studies.

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FOOTNOTES

Contributors NT conceived study design and participated in protocol development, literature searching, data extraction, data analysis and drafted the manuscript. MH

critically reviewed the draft and contributed to the manuscript revisions.

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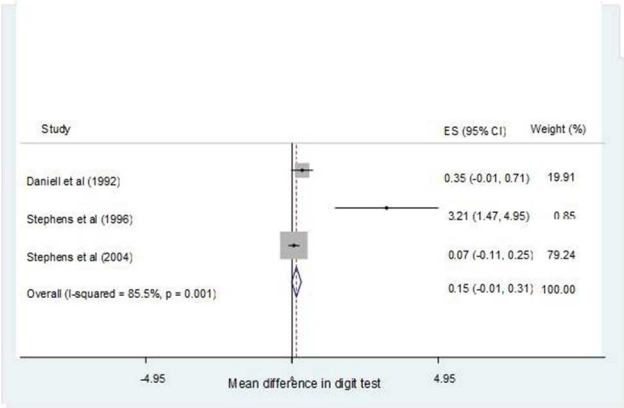


Figure1 shows the result of meta-analysis using NES
254x190mm (96 x 96 DPI)

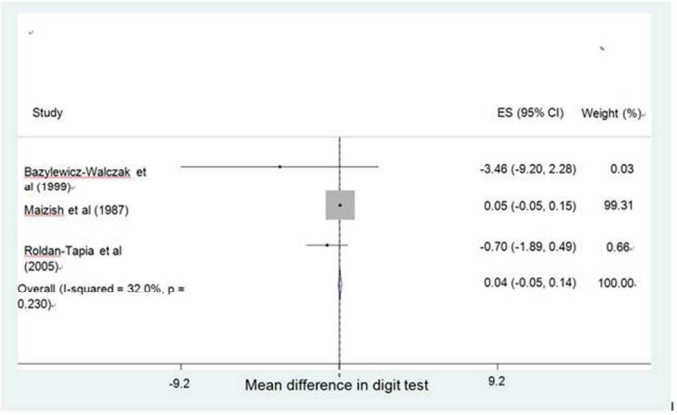
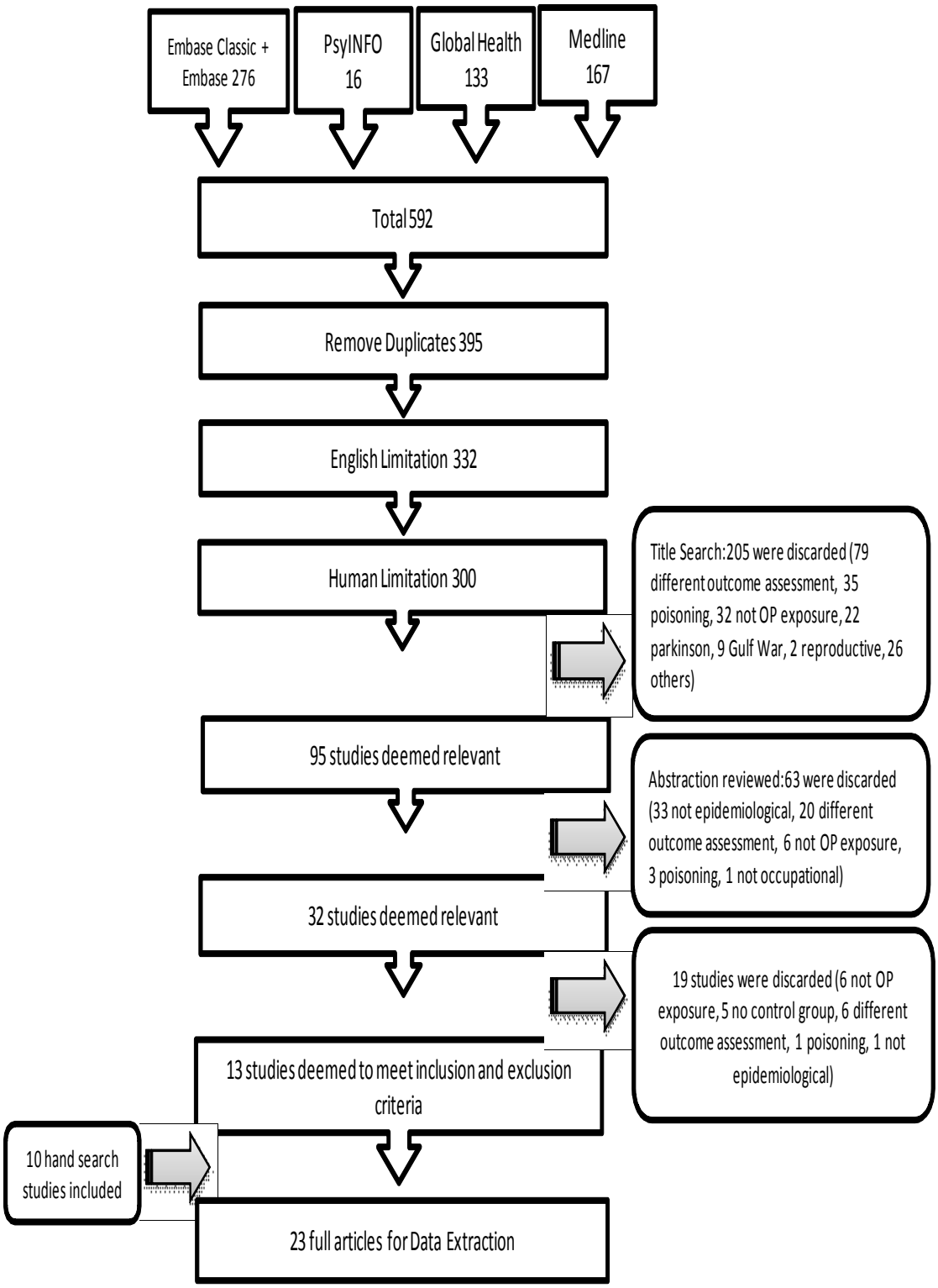


Figure2 shows the result of meta-analysis using WAIS.
254x190mm (96 x 96 DPI)

Appendix A

The flow of study inclusion and exclusion diagram



Appendix B

The Appraisal Standard of Newcastle/Ottawa Scale

Selection

1) Representativeness of the exposed group/cohort

a) Truly representative of the average farmers or pesticides applicators in the community *

b) Somewhat representative of the average farmers or pesticides applicators in the community*

c) Selected group of users (e.g. factory workers, volunteers)

d) No description of the derivation of the group

2) Selection of the non-exposed group/cohort

a) Drawn from the same community as the exposed group*

b) Drawn from a different source

c) No description of the derivation of the non-exposed group

3) Ascertainment of exposure

a) Secure record (e.g. biomarkers)*

b) Structured interview or questionnaire*

c) Written self reports

d) No description

4) Demonstration that outcome of interest was not present at start of study *(Cohort Studies Only)*

a) Yes*

b) No

Confounder

1) Comparability of groups on the basis of the design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor* (e.g. alcohol consumption, smoking, and first language)

Outcome

1) Assessment of outcome

a) Independent blind assessment*

b) Record linkage*

c) Self reports

d) No description

2) Was follow-up long enough for outcomes to occur *(Cohort Studies Only)*

a) Yes (select an adequate follow up period for outcome of interest)*

b) No

3) Adequacy of follow up of cohorts *(Cohort Studies Only)*

a) Complete fellow up – all subjects accounted for*

b) Subjects lost to follow up unlikely to introduce bias – small number lost - > 70% follow up, or description provided of those lost*

c) Follow up < 70% and no description of those lost

d) No statement

Case Control Studies:

Selection

1) Is the case definition adequate?

a) Yes, with independent validation*

b) Yes, e.g. record linkage on self reports

c) No description

2) Representativeness of the cases

a) Consecutive or obviously representative series of cases*

b) Potential for selection biases or non stated

3) Selection of Controls

a) Community controls*

b) Hospital controls

c) No description

4) Definition of Controls

a) No history of disease (endpoint)*

b) No description of source

Confounder

1) Comparability of cases and controls on the basis of design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor*

Exposure

1) Ascertainment of exposure
a) Secure record (e.g. biomarkers)*
b) Structured interview where blind to case/control status*
c) Interview not blinded to case/ control status
d) Written self reports or medical record only
e) No description

2) Same method of ascertainment for cases and controls
a) Yes*
b) No

3) Non-Response rate
a) Same rate for both groups*
b) Non respondents described
c) Rate different and no designation
*: plus one point

There are five items in cross-sectional studies and eight items in cohort and case control studies, respectively. The quality of the studies was defined as follows.

Cross-sectional Studies:
Very Good Studies: 5 points
Good Studies: 4 points
Satisfactory Studies: 3 points
Unsatisfactory Studies: 0 to 2 points

Cohort / Case control Studies:
Very Good Studies: 7 to 8 points
Good Studies: 5 to 6 points
Satisfactory: 4 points
Unsatisfactory Studies: 0 to 3 points

Appendix C

Table1 Quality Appraisal (Cross-sectional Studies)

Selection	Cole et al 1997	Dassanayake et al 2009	Farahat et al 2003	Fiedler et al 1997	Korsak et al 1977	Levin et al 1976
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	a) (+1)	b) (+1)	c) (0)	a) (+1)	b) (+1)	b) (+1)
2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	b) (0)	b) (0)	a) (+1)	a) (+1)	b) (0)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	d) (0)	a) (+1)	b) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and	b) (+1)	- (0)	a) (+1)	- (0)	- (0)	- (0)

education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)						
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	a) (+1)	b) (+1)	d) (0)	b) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	2/5 Unsatisfactory	2/5 Unsatisfactory	4/5 Good	3/5 Satisfactory	3/5 Satisfactory

Continued…

Table1 Continued

Selection	London et al 1997	London et al 1998	Maizish et al 1987	Rodnitzky et l 1975	Roldan-Tapia et al 2005
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	b) (+1)	a) (+1)	c) (0)	c) (0)	a) (+1)
2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	c) (0)	a) (+1)

3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	b) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	b) (+1)	b) (+1)	b) (+1)	- (0)	a) (+1)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	c) (0)	a) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	4/5 Good	4/5 Good	1/5 Unsatisfactory	5/5 Very Good

Continued…

Table1 Continued

Selection	Srivastava et al 2000	Steenland et al 2000	Stephens et al 1995	Stephens et al 1996	Stephens et al 2004
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	c) (0)	a) (+1)	a) (+1)	a) (+1)	a) (+1)

2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	a) (+1)	a) (+1)	c) (0)	a) (+1)	b) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	- (0)	b) (+1)	b) (+1)	b) (+1)	- (0)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	d) (0)	a) (+1)	b) (+1)	d) (0)	b) (+1)
Overall Score	2/5 Unsatisfactory	5/5 Very Good	4/5 Good	4/5 Good	4/5 Good

Table2 Quality Appraisal (Cohort Studies)

Selection	Albers et al 2004	Bazylewicz-Walczak et al 1999	Daniell et al 1992	Ohayo-Mitoko et al 2000	Misra et al 1985	Ross et al 2010
1) Representativeness of the exposed cohort a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the cohort	c) (0)	c) (0)	a) (+1)	b) (+1)	c) (0)	a) (+1)
2) Selection of the non exposed cohort a)Drawn from the same community as the exposed cohort b)Drawn from a different source c) No description of the derivation of the non exposed cohort	b) (0)	a) (+1)	b) (0)	a) (+1)	b) (0)	b) (0)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	a) (+1)	a) (+1)	a) (+1)	b) (+1)	a) (+1)	b) (+1)
4)Demonstration that outcome of interest was not present at start of study a) Yes b) No	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	- (0)	a) (+1)	b) (+1)	- (0)	a) (+1)	a) (+1)

Continued…

Table2 Continued

Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	d) (0)	d) (0)	c) (0)	d) (0)	d) (0)
2) Was follow-up long enough for outcomes to occur a) Yes (select adequate follow up period for outcome of interest b) No	b) (0)	b) (0)	b) (0)	b) (0)	b) (0)	a) (+1)
3) Adequacy of follow up of cohorts a) Complete follow up-all subjects accounted for b) Subjects lost to follow up unlikely to introduce bias- small number lost- >70% follow up, or description provided of those lost c) Follow up rate<70% and no description of those lost d) No statement	b) (+1)	a) (+1)	a) (+1)	c) (0)	d) (0)	d) (0)
Overall Score	4/8 Satisfactory	5/8 Good	5/8 Good	4/8 Satisfactory	3/8 Unsatisfactory	5/8 Good

Table3 Quality Appraisal (Case-control Studies)

Selection	Beseler et al 2006
1) Is the case definition adequate?	
a) Yes, with independent validation b) Yes, e.g. record linkage or based on self reports C) No description	b) (0)
2) Representativeness of the cases	
a) Consecutive or obviously representative series of cases b) Potential for selection biases or not stated	a) (+1)
3) Selection of Controls	
a) Community controls b) Hospital controls C) No description	a) (+1)
4) Definition of Controls	
a) No history of disease (endpoint) b) No description of source	a) (+1)
Confounders 1) Comparability of cases and controls on the basis of design or analysis	b) (+1)
a) Study control for age and education b) Study controls for any additional factor	
Exposure 1) Ascertainment of exposure	
a) Secure record(biomarkers) b)Structured interview where blind to case/control status c) Interview not blinded to case/control status d) Written self report or medical record only e) No description	d) (0)

Continued…

Table3 Continued

2) Same method of ascertainment for cases and controls a) Yes b) No	a) Yes
3) Non-response rate a) Same rate for both groups b) Non respondents described c) Rate different and no designation	b) (0)
Overall Score	5/8 Good

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A systematic review of the influence of occupational organophosphate pesticides exposure on neurologic impairment

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Authors:

Noriko Takahashi

Masahiro Hashizume

Authors' Institutions:

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Address for correspondence:

Noriko Takahashi

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Tel: (+81) 95 819 7764 Fax: (+81) 95 819 7844

E-mail: pediatric.nagasaki@gmail.com

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ABSTRACT

Objective: The aim of this study was to conduct a systematic review of published literature and to estimate whether or not there is a causal relationship between occupational exposure to Organophosphate pesticides (OPs) and either neurologic impairment or depressive symptoms.

Data sources: EMBASE, MEDLINE, Global Health, and PsycINFO (1980 to April 2014).

Setting: Observational studies (cross-sectional, cohort, and case-control studies) with both exposed and unexposed groups.

Participants: People who occupationally use OPs more than one month and their family.

Primary outcome: Results of neurological core test batteries or depressive symptoms such as headaches, anxiety, and dizziness.

Study appraisal and synthesis methods: After an extensive search of various literature databases, one author screened titles and abstracts, searched the relevant publications manually, and conducted data extraction. All extracted data from the selected articles were synthesized for analysis. Quality appraisal was conducted using Newcastle Ottawa Scale.

Results: Of the 1024 articles retrieved by database search, 24 studies that met inclusion and exclusion criteria were selected for analysis. Of the selected studies, 17 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10 studies), UK (four studies), Africa (four studies), Asia (three studies), Europe (two studies), and South America (one study). Each of the included studies used different exposure

and outcome assessments such as neurologic scores and depressive symptoms, making it difficult to compare the results exactly. Most studies showed that exposed groups had poorer results than unexposed groups; however, because of inconsistent neurological test batteries there was not enough pooling evidence to conduct a meta-analysis.

Conclusion: The findings of this literature review indicate that it is a necessary to standardize the neurological or neuropsychological test battery and methods of measuring exposure to OPs.

Trial registration: Not applicable.

ARTICLE SUMMARY

Article Focus

- To systematically review epidemiological studies that examine adverse effects on the human central nervous system (CNS) by exposure to organophosphate pesticides (OPs).

Key messages

- OPs have been used widely all over the world for agricultural or industrial use.
- Many studies have examined acute health problems caused by OPs; however, few studies have investigated negative effects caused by occupational OPs exposure.

Strengths and limitations of this study

- The article represents a systematic review of epidemiological studies on adverse effects on the human CNS by occupational OPs exposure, with a quality appraisal of each study.
- The article identifies problematic issues of exposure and outcome assessments.
- Meta-analysis could not be applied because only a small number of pooled studies were available.
- In some studies it was difficult to judge negative effects caused only by OPs, because mixed pesticides were used.

INTRODUCTION

Ever since organophosphate pesticides (OPs) were developed, they have been used to combat insects for public health purposes and to support agricultural productivity and manufacturing processes. Because pesticides are also one of the leading suicide methods, a large number of epidemiological studies have investigated the relationship between high level OPs exposure such as pesticide poisoning and accidents and acute health effects. It has been reported that high level OPs exposure is significantly related to neurological or neuropsychological impairment (1, 2). In contrast, few studies have reported associations between occupational or cumulative OPs exposure and negative effects on human health, although some research has examined the negative influence on young children of cumulative OPs exposure (3, 4) and others have investigated relationships between reproductive health and occupational OPs exposure (5-7). High level OPs exposure are known to have adverse effects on the human CNS, therefore, occupational or cumulative OPs exposure also has the potential to negatively affect the CNS. However, very few epidemiological studies that have assessed the relationships between occupational OPs exposure and neurologic or mental problems have been published. The objective of this systematic review is to verify whether or not occupational OPs exposure negatively affects the human CNS. To investigate this further, we summarized the epidemiological evidence for the relationship between occupational OPs exposure and mental and neuropsychological aggression, especially for occupational OP users, and some of the limitations associated with the various studies are discussed.

MATERIALS AND METHODS

Searching strategy for identification of published studies

We searched the published literature using the OvidSP search software (8) to select relevant observational studies. A geographical restriction was not imposed; however, the search was restricted to studies published from 1980 to 2014. Population-based case-control studies were excluded from the systematic review because it was difficult to assess accurate exposure doses for these studies. Because various pesticides including OPs are currently easily available to everyone, it is highly likely that these pesticides have been obtained for personal use. For this reason, it is almost impossible to obtain past records of pesticide use by every individual. The literature search was limited to studies in humans and to reports published in English, and the review was limited to epidemiological studies. Moreover, unpublished studies and grey literature (literature that has not been formally published) were not searched in this systematic review; therefore we did not contact authors to find unpublished studies. Studies investigating OPs exposure through food and water contamination were also excluded. A search of the following four databases was carried out: EMBASE Classic + EMBASE (1980 to Week13 2014); Ovid MEDLINE(R) (1980 to Week13 2014); Global Health (1980 to Week12 2014); and PsycINFO (1980 to Week14 2014).

A combination of free-text terms and explore terms was used to identify relevant articles. For exposure, the following search keywords were used: organophosphate*, organophosphorous,

pesticide*, or insecticide*, and organophosphate pesticide (explore map term). For outcome, the following search keywords were used: neuro*, psychiatr*, psycholog*, mental health, mental illness, mental disorder, or depressi*, depression (explore map term), and mental health (explore map term). For subjects, the following search keywords were used: occupation*, agricultu*, or farm*. For study design, the following search keywords were used: epidemiolog*, cohort, cross-sectional, or case-control, and epidemiology (explore map term). An initial systematic search in the titles and abstracts was conducted using a combination of all these search terms. A second manual search of the reference lists from the selected relevant articles was performed to explore or retrieve articles found in the initial search in order to find as many available studies as possible.

Criteria for selecting studies for review

Only original research articles meeting the inclusion and exclusion criteria described below were used in the final review.

Inclusion criteria:

1. Study design

a) Must be observational studies: cross-sectional, cohort, and case-control studies.

b) Studies must have both exposed and unexposed groups.

2. Subjects

a) The subjects in the exposed group either must use OPs occupationally, or there must be a probability of being exposed to OPs during their work.

b) The families of occupational OP users can be treated as subjects.

3. Exposure

a) Subjects must be exposed to OPs for at least one month.

b) Seasonal workers who used OPs for more than one month must be included.

4. Outcome

Studies must have carried out some tests to assess damage to the CNS or have conducted a survey or an interview to identify depressive symptoms.

5. Exposure-outcome association

Results must be reported as some types of relative risks or mean scores.

Exclusion criteria:

1. Study design

Experimental and laboratory based studies including animal studies were excluded. Population-based case-control studies were excluded.

2. Subjects

Studies of mainly patients of pesticide poisoning were excluded.

3. Exposure

Studies that did not specify the type of pesticides were excluded.

4. Outcome

Studies examining damage of the peripheral nervous system due to OPs exposure were excluded.

5. Language

Studies published in a language other than English were excluded.

Definitions used for the review

Definition of cumulative exposure

a) People who used OPs in their jobs for at least one month and had the probability of inhaling ambient OPs and absorbing OPs by spraying and touching.

b) Families of OP users were included as subjects because they may have been exposed to OPs by washing clothes contaminated by OPs and/or by touching OP users.

Definition of poor mental health

A) Neurological or neuropsychological impairment

a) People who had poorer results in neurological or neuropsychological test batteries than healthy people of the same age.

b) People who had short-memory loss; for example, people who had experienced memory loss of six to three months duration.

B) Depressive Symptom

c) People who, regardless of their age, had chronic depressive symptoms including headache, fatigue, dizziness, sleepless, and eye problems.

d) People who were diagnosed with depression by clinical doctors.

Study selection process

Using the search terms listed above, a total of 1024 references were obtained: 515 from EMBASE Classic + EMBASE, 31 from PsycINFO, 196 from Global Health, and 282 from Ovid MEDLINE(R) (Figure1). However, 77 animal studies, 90 studies not in English studies, and 12 studies that did not meet the time restrictions were excluded. Of the remaining 845 studies, 516 were excluded because of duplications. A manual search of the titles and abstracts of the remaining 329 references excluded a further 272 studies. The 21 remaining articles were fully reviewed, after which 12 studies were deemed to meet inclusion and exclusion criteria (9-20). In addition, 12 articles identified by the manual search were added to the systematic review (Figure1). To include as many relevant studies as possible, studies published before 1980 that were found by the manual search were included to the list for review. Finally, these 24 studies were selected for data extraction (9-32).

Data extraction, synthesis, and analysis

Data extraction forms were created to compare relevant data collected from each of the 24 studies. The following data were extracted to assess heterogeneity of the included studies: title, authors, year

published, number of subjects in the exposed and unexposed groups, occupation, and demographic information such as mean age, sex, smoking status, and geographical area. In addition, the following data were extracted to assess confounding factors and statistical models among the included studies: inclusion and exclusion criteria such as first language, alcohol consumption, injury experience, confounding factors, and statistical methods used. The following data were extracted to assess exposure and outcomes: types of pesticides, exposure assessment, and outcome assessment to measure the neurologic or neuropsychological ability, and results obtained. Tables containing the data that were obtained using the data extraction forms were constructed and analyzed. P-values and 95% confidence intervals (95%CIs) were elicited from the articles to judge statistical uncertainty. When a study had investigated depressive symptoms, the information was collected and a table was constructed. Impact and statistical magnitude of depressive symptoms were represented using plus or minus signs '++', '+', and '−', based on the P-value or 95%CI of the studies. All data extraction, coding, and quality appraisal were conducted only by the first author; therefore, no disagreement events occurred.

Quality appraisal

The quality of the 24 studies was appraised using a scale adapted from the 'Newcastle/Ottawa Scale (NOS)'(33) (The appraisal standard of NOS is shown in Appendix A). Based on the NOS, each study was evaluated using the point system. When a study included relevant information that could be

associated to the NOS, one point was added. Five items in cross-sectional studies and eight items in cohort and case-control studies that could be related to the NOS were identified. Therefore, cross-sectional studies assigned 5, 4, 3, or 0−2 points were evaluated as very good, good, satisfactory, or unsatisfactory studies, respectively. Similarly, cohort/case-control studies with 7−8, 5−6, 4, and 0−3 points were identified as very good, good, satisfactory, or unsatisfactory, respectively.

RESULTS

As a result of the search strategy described in the Materials and Methods section, 12 studies were identified from the database search and another 12 studies were found after a manual search. These 24 articles, published between 1975 and 2010, met all the inclusion and exclusion criteria. A summary of the characteristics of the 24 selected articles is shown in Table 1.

Study design and geographical area

Of the selected studies, 17 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10 studies), UK (four studies), Africa (four studies; two in South Africa, one in Egypt, and one in Kenya), Asia (three studies; two in India and one in Sri Lanka), Europe (two studies; one in Spain and one in Poland), and South America (one study; Ecuador).

Characteristics of subjects

Because the subjects were limited to people who had the probability of being occupationally exposed by OPs, the majority of the participants (60−70%) were men. Most of the time, agricultural work such as pesticide application and farming is performed predominantly by men. Six of the 24 studies included both male and female subjects (9, 11, 17, 25, 27, 32), and only one study used all female subjects in both the exposed and control groups (21). In 13 of the studies the mean age of the exposed subjects was in the 30s, in six studies the mean age was in the 40s (9, 14, 15, 19, 20, 31), and in two studies the mean age was in the 50s (13, 17). The mean age in two studies was 29, very close to thirty (25, 32). One of the studies did not report detailed demographic data of the participants (10).

Source of recruitment and sample size

Ten out of the 24 studies were on pesticide applicators including private, commercial, and tree, fruit, and vegetable applicators. Five and three studies were on farmers and sheep farmers, respectively, and two studies were on factory workers and greenhouse workers. One study investigated depressive symptoms in the spouses of OPs users. In the study by Korsak et al. (22), the specific occupation of the population in the study was not stated, however, the subjects had experienced occupational OPs exposure. The number of subjects in the exposed groups varied from 16 to 2,051, while the control groups had a wider range of subjects (16 to 27,023).

Table1 Findings reported in epidemiological studies into occupational low level OPs exposure and mental illness

	Author	Study Design	Country	Exposed Population(No)	Chemical	Exposed Assessment	Comparison Group
1	Albers et al (9)	CO	USA	Chemical workers(53)	OP	Industrial HR,AChE INH	Similar workers, not exposed(60)
2	Bazylewicz-Walczak et al (21)	CO	Poland	Greenhouse workers(26)	OP	DR	Greenhouse workers, not exposed(25)
3	Beseler et al (10)*	NC/ CO	USA	Case**: Spouses of private applicators with depressive diagnoses(2,051)	OP	QU or IN	Control: Spouses of private applicators without depressive diagnoses (27,023)
4	Cole et al (11)	CR	Ecuador	Farmers, some applicators(144)	OP,CAR, FNG	IN, QU, AChE INH	Local Population(72)
5	Daniell et al (12)	CO	USA	Farm worker applicators(49)	OP	QU, AChE INH	Slaughterhouse workers(40)
6	Dassanayake et al (13)	CR	Sri Lanka	Vegetable farm workers (38)	OP	N.A.	hospital labours(35)
7	Farahat et al (14)	CR	Egypt	Farm workers(52)	OP	AChE INH	Local Population(50)
8	Fiedler et al (15)	CR	USA	Tree fruit farmers (57)	OP	QU, lifetime exposure metric	Cranbury/blueberry growers(low exposed), hardware storeowners(unexposed) (42)
9	Korsak et al (22)	CR	USA	Occupational exposure(16)	OP, CAR, OC	AChE INH	Local Population(low exposure)(16)
10	Levin et al (23)*	CR	USA	Pesticide applicators(24)	OP	IN, AChE INH	Farmers(24)
11	London et al (16)	CR	South Africa	Fruit farm pesticide applicators(163)	OP	QU(job-matrix)	Farm workers, not applicators(84)
12	London et al(24)*	CR	South Africa	Fruit farm pesticide applicators(164)	OP	QU (job-matrix)	Farm workers, not applicators(83)
13	Maizlish et al (25)	CR	USA	Pesticide applicators(46)	OP	UM, DR	Non-applicators(56)
14	Misra et al (26)*	PR	India	Pesticide applicators(22)	OP	AChE INH	Hospital labours(20)

15	Ohayo-Mitoko et al (27)*	CO	Kenya	Farm worker applicators(256)	OP, CAR	AChE INH	Farm workers(low exposure)(152)
16	Rodnitzky et al (28)	CR	USA	Pesticide applicators(23)	OP	AChE INH	Farmers(23)
17	Roldan-Tapia et al (18)	CR	Spain	Greenhouse workers(40)	OP, CAR	QU, AChE INH	Local Population(26)
18	Ross et al (17)	CO	UK	Sheep farmers(127)	OP	IN	Police workers(78)
19	Rothlein et al(32)	CR	USA	Farm workers(96)	OP	UM, House dust	Workers in hotels and tourist industry(45)
20	Srivastava et al(29)	CR	India	Manufacture workers(59)	OP	AChE INH	Manufacture workers, not exposed(17)
21	Steenland et al(30)	CR	USA	Termiticide applicators(191)	OP	IN,UM	Friends, blue collar workers(189)
22	Stephens et al (19)	CR	UK	Sheep farmers(146)	OP	QU	Quarry workers(143)
23	Stephens et al (31)	CR	UK	Sheep farmers(77)	OP	QU, UM	Quarry workers(69)
24	Stephens et al (20)	CR	UK	Orchard applicators(37)	OP	IN,QU	Construction workers,pig farmers(57)

Study Design CR: Cross-sectional, CO: Cohort, NC: Nested case-control, PR: Prospective study

Chemical OP: Organophosphates, OC: Organochlorines, CAR: Carbamates, FUN: Fungicides, AChE: Acetylcholinesterase

Exposed Assessment AChE INH: AChE inhibition, DR: Dermal and respiratory absorption, IN: Interview , QU: Questionnaire , HR: Hygiene records UM: Urinary metabolites

*Studies that included depressive symptoms for outcome assessments.

**Cases were defined as female spouses of private applicators who responded 'yes' to the question "Has a DOCTOR ever told you that you had been diagnosed with depression requiring medication?" Controls were female spouses who responded 'no' (10).

Exposure assessment

Exposure assessment in the included studies could be divided, for the most part, into five patterns: indirect assessment using, for example, an interview or questionnaire; direct assessment including a measurement of urinary metabolites and acetylcholinesterase (AChE) levels in the blood or a measurement of ambient OPs using a patch and a pump; combination of direct and indirect methods; combination of a biomarker and OPs exposure levels included in house dust; and combination of biomarkers and ambient OP levels. Seven of the 24 studies used indirect methods, and six studies used blood AChE inhibition levels to measure AChE levels in the blood as an exposure indicator. Six studies used a combination of indirect methods and biomarkers, three studies used biomarkers and the ambient OP levels, one study used a biomarker and house dust. The remaining study did not mention any exposure assessment methods. In all the studies that used urinary metabolites as exposure assessment, the results were presented as the sum of dialkylphosphates (DAP) (i.e. the sum of six DAP metabolites: DMP (dimethylphosphate), DMTP (dimethylthiophosphate), DMDTP (dimethyldithiophosphate), DEP (diethylphosphate), DETP (diethylthiophosphate), and DEDTP (diethyldithiophosphate)) (25, 30-32).

Outcome measurements

Two different outcome measurements were used in the studies; one measured neurological impairment and the other assessed depressive symptoms. Of the 24 studies, 19 used cognitive function tests to investigate negative neurologic influences caused by OPs exposure.

Associations between outcome and exposure

Ten of the 19 studies that investigated cognitive impairment mentioned that at least one measure outcome showed more impairment in the exposed groups; however, these observations were not significant ($P<0.05$). Seven of the studies reported some significant positive associations of exposure with poor outcome ($P <0.05$); however, even in these cases, the significant decrements were observed only in some of the neurologic tests, mainly in the Digit Span and Santa Ana Dexterity tests. Indeed, there are several versions of these neurologic tests and the significance of the scores often depended on the versions of the tests that were used. Five studies used the Wechsler Adult Intelligence Scale (WAIS or WAIS-R) (34, 35), four studies used the Neurobehavioral Evaluation System (NES) (36), two studies used the World Health Organization Neurobehavioral Core Test Battery (NCTB) (37, 38), and the remaining

eight studies used their own scales.

Five studies adopted depressive symptoms as outcome measurements, as shown in Table2; however, the symptoms used in the studies were not standardized.

Table2 Summary of depressive symptoms used as outcomemeasurements

Reference	Results obtained	Impact of outcomes
Beseler et al 2006(10)	Depression due to doctor's diagnosis was not significantly related to low (OR 1.09; 95%CI 0.91, 1.31) or high (OR 1.09; 95%CI 0.91, 1.31) cumulative exposure.	-
Levin et al 1976(23)	Anxiety score of the pesticide applicators was significantly higher (P<0.05) than that of the farmers. However, there was no significant difference in measures of depression.	++
London et al 1998(24)	Dizziness, sleepiness, and headache had a significantly higher overall neurological symptom score (P<0.05).	++
Misra et al 1985(26)	Common symptoms were Headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%) and no neurologic change was seen.	-
Ohayo-Mitoko et al 2000(27)	A significant change in symptom prevalence was found for the respiratory (2.48% CI (0.78, 5.38) and central nervous system (2.56% CI (0.99, 6.62), but in terms of skin symptoms, and eye symptoms, there was no statistically significant change.	++

OR=Odds Ratio ++: Statistically significant (P<0.05), -: Not statistically significant

Statistical analysis

Sixteen studies used logistic regression, and the remaining eight used other statistical tests including X^2-test and t-test. Only one study adjusted for sex in the logistic

regression. Fourteen of the 24 studies adjusted for age, and 12 adjusted for education in the statistical analysis. However, only five studies adjusted for alcohol consumption before carrying out the statistical analysis, and only two studies adjusted for first language.

Methodological quality appraisal

Based on NOS, five of the 24 studies were of very good quality, 10 were of good quality, and the remaining nine were either satisfactory or unsatisfactory. Most studies with unsatisfactory scores either were carried out before 1990 or were performed in some of the less developed countries. In particular, the methods of recruitment of subjects, controlling for confounders, and outcome assessment were not appropriate. For example, in some studies, all of the participants were volunteers (14, 28) and in another study, the subjects were not representative of the community from which they were recruited (factory workers) (29). In addition, in the unsatisfactory studies, how the outcome was assessed was not described, and methods needed to avoid confounders such as stratification and regression were not used. None of the cohort studies were assessed as very good quality because most of them did not have a long enough follow-up duration (in five studies, the duration was less than six months) and the

selected subjects were not fully representative of the target community. Moreover, the methods of outcome assessment were not described in most of the cohort studies.

Data synthesis

The results of the neurologic tests used in the studies are summarized in Table3. As can be seen, the test batteries differed from study to study. The commonly used test batteries in NCTB, NES, and WAIS were Symbol-Digit and Digit Span Forward and Backward. However, some studies that adopted NES and WAIS to measure neurologic impairment implemented only a few subsets in the trials. Among the 13 studies that used a Symbol-Digit test, four used NES and unknown tests, two used WAIS and WAIS-R, and one used a Polish NCTB. Among the studies that used Digit Span Forward and Backward tests, some studies performed both tests, while the others did only one of the tests as shown in Table3. Overall, only four of the studies used the same test battery in NES and WAIS. Although three studies apparently used the same scoring systems, one of the scores was completely different from the scores in the other two studies. For example, the scores in the study by Stephens et al. (31) were 24.22 and 21.01 in the exposed and the control groups respectively, whereas the scores reported by Daniell et al. and Stephens et al. were much lower and between 2.23 and 3.55 (12, 20). Similarly,

the mean scores reported by Bazylewicz-Walczak et al. (21) were higher, 45.50 and 49.40, while the mean scores reported in the other studies were smaller, 2.28 and 2.23 in the WAIS (25). In consideration of insufficient number of studies and possible systematic differences in the population characteristics and/or in the measurement procedures between the studies, we decided not to conduct a meta-analysis.

Table3 Summary of the neurologic test batteries used in some of the studies

Reference	Types of neurologic tests	Symbol Digit	Digit Span	Santa Ana	Simple Reaction Time	Syntactic Reasoning(s)
Bazylewicz-Walczak et al 1999(21)	Polish NCTB/WAIS (Symbol Degit)	nd	nd	nd	**	nd
Cole et al 1997(11)	NCTB	nm	nm	nm	nd	nd
Daniell et al 1992(12)	NES	*	nd	nd	nd	nd
Farahat et al 2003(14)	Unknown	***	***(f)[1] ***(b)[2]	nd	nd	nd
Fiedler et al 1997(15)	WAIS-R	*	*	nd	***	nd
London et al 1997(16)	WAIS-R	nm	nm	***	nm	nd
Maizlish et al 1987(25)	WAIS	***	nd	nd	nd	nd
Roldan-Tapia et al 2005(18)	WAIS	*** [3]	*** [3]	nd	nd	nd
Ross et al 2010(17)	WAIS	nd	***	nd	nd	nd
Rothlein et al 2006(32)	Unknown	*	*(f)[1] ***(b)[2]	nd	*	nd
Srivastava et al 2000(29)	Unknown	***	***	nd	nd	nd
Steenland et al 2000(30)	NES	*	*	nd	*	nd
Stephens et al 1995(19)	Unknown	***	*	nd	***	**
Stephens et al 1996(31)	NES/ACT	nm	nm	nd	nm	nm

Stephens et al 2004(20)	NES/ACT	*	*	nd	*	*** (ACTS)

***P<0.05, **0.05≦P<0.1, *P>0.1

The exposed groups were slower or had poorer outcomes than the control groups

[1](f) Digit Span Forward

[2](b) Digit Span Backward

[3]Whether the obtained results were positive or negative was not reported in the study.

nd: Subsets of neurological tests were not performed.

nm: Subsets of neurological tests were performed but P-values were not reported.

DISCUSSION

The systematic keyword and manual searches of the published literature identified 24 epidemiological studies that examined the relationship between OPs and CNS. When the relevant information was assessed, two main findings were obtained, one was the method of exposure assessment, and the other was the method used for the outcome measurement. For exposure assessment, the measurement methods were categorized as direct, indirect, and a combination of direct and indirect. For the outcome measurements, two main assessments were used, neurologic impairment and depressive symptoms.

Exposure assessment

Exposure assessment was not used for group allocation in all the studies; rather, it was implemented to measure how much subjects were exposed and the outcomes of the neurobehavioral tests. Different exposure assessment methods were used in each study,

which made it difficult to accurately compare the studies. In addition, there seemed to be methodological imperfections in both the direct and indirect methods. For example, in one study, an interview and questionnaire were used for recruited subjects over 60 years old who had been retired for 11 years (17). This method is subject to recall bias because the rate of cognitive impairment is likely to have increased as the subjects aged. However, other indirect methods, especially extensive history records of pesticide use could be considered as a proxy of how much OPs might have accumulated in the body, thus records of this type can be used to estimate the amount of OPs by long-term exposure, even though there may be some recall bias. For the direct methods, DPA or urinary metabolites was used as an exposure index in the study; however, DPA is metabolized rapidly and excreted (6). On the contrary, blood AChE levels take approximately one week to become normal (39); hence, although blood AChE levels cannot be used to assess the accumulation of OPs in body tissues over a long time, it can be used to assess short-term exposure. To minimize measurement errors, a mixed method for the assessment of short-term and long-term exposure should be established.

Outcome assessment

The main problem in analyzing the outcome measurements was the inconsistencies in

neurologic test batteries. Various versions of the neurologic tests were used in the studies and the content of the tests differ slightly in each study (Table3). Therefore, only a few tests were common across some of the studies, which made it difficult to compare the studies. Further, a meta-analysis could not be applied because of the insufficient number of studies. Meta-analysis could have been performed by dividing the results into subgroups; however, the results could be highly misleading because of loss of power (40). In terms of depressive symptoms, the outcome assessment was again different in each study. For instance, one study used the proportion of headaches, while another used dizziness and sleepiness as the main outcomes. To gain better insights into whether occupational OP exposure can negatively affect the human CNS, at the very least, neurologic test batteries should be standardized and guidelines for measuring of neurologic symptoms should be set for all future epidemiological studies. Furthermore, although some studies mentioned the possible relationship between OPs exposure and confounding factors such as age and education, statistical tests between the exposed and control groups were not performed in these studies. These inconsistencies make it difficult to compare the neurologic impairment outcomes among the studies.

Study design

Although 17 of 24 studies were cross-sectional studies, longitudinal or cohort studies are more appropriate, because agricultural work using pesticides is easily influenced by seasonality. One research regarding reproductive health by OPs exposure stated that sperm concentration and counts are negatively affected in spring, peak season, rather than winter (5). Therefore, the effect on the CNS could also be affected by seasonality.

Sources of possible biases

Only published studies written in English were searched, thus publication bias could have occurred. In future studies, non-English studies and unpublished studies should be included to reduce publication bias. In trials that included foreign workers, first language and education levels could be considered as possible biases because there is a possibility that non-native subjects did not fully understand the content and instructions for the tests, which could lead to them obtaining a lower score than native speakers. Additionally, the education systems in developed and less developed countries could be very different. Nowadays, developed countries such as USA and the Gulf countries have accepted foreign workers as an important part of the workforce (12, 32, 41). These factors needed to be adjusted carefully in the sampling and analytical stages of the study; however, only two of the selected studies mentioned first language in their

statistical analyses (12, 31). Occupation could also contribute to selection bias because, for example, a police officer or a construction worker would have a higher probability of experiencing loss of consciousness due to accidents than workers with different occupations (17).

Possible confounders

Apart from common confounders such as age and education, head injury and alcohol consumption could be other confounders, because they can cause neurologic impairment due to memory deterioration. Although some of the studies adjusted for alcohol consumption in the analysis (10, 12, 16, 24, 25), no study adjusted for head injury. Furthermore, nutrition status including vitamin deficiency can also be relevant to the outcome of neuropsychological tests (16, 24). Thus, factors other than the common confounders that could negatively affect cognitive function should be adjusted for in the analysis.

Strengths and limitations of this review

A major strength of this systematic review is that the characteristics of the selected studies were summarized using tables, and limitations of the exposure and outcome

assessments used in these studies were identified mainly on the basis of the constructed tables. Furthermore, the systematic review allowed us to propose recommendations that will be useful for standardizing future epidemiological research.

All of the selected studies were relevant to occupational OPs exposure; however, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides that are commonly used in agriculture are usually mixtures of different pesticides, which are used to increase their effect. Four of the 24 selected studies used a combination of OPs, organochlorines, carbamates, and fungicide; hence, the effect of only occupational OPs exposure could not be measured in these studies. In the outcome assessments, different neurological types of tests were used, consequently, the lack of pooling evidence meant that a meta-analysis could not be performed. Furthermore, the exclusion of studies written in languages other than English is another limitation of this review.

CONCLUSION

The items tested in the neurological or neuropsychological test batteries, and the estimates of OPs exposure were inconsistent because they depended on the preferences of the investigators. For future studies, the neurological and neuropsychological test

types, test batteries, and the methods used to measure OPs should be standardized to ensure adequate quality and to make it possible to pool the evidence from a large number of studies for future analysis.

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FOOTNOTES

Contributors NT conceived the study design and participated in protocol development, literature searching, data extraction, data analysis, and drafted the manuscript. MH critically reviewed the draft and contributed to the manuscript revisions.

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FIGURE LEGEND

Figure 1: Flow diagram of search and review process

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Title: A systematic review of the influence of occupational organophosphate pesticides exposure on neurologic impairment

Authors:

Noriko Takahashi[†]

Masahiro Hashizume[†]

Authors' Institutions:

[†] Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, ~~Nagasaki City,~~ Nagasaki~~,~~ 852-8523, Japan

Address for correspondence:

Noriko Takahashi~~Furuoya~~

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

1-12-4 Sakamoto, ~~Nagasaki City,~~ Nagasaki 852-8523, Japan

Tel: (+81) 95 819 7764 Fax: (+81) 95 819 7844

E-mail: pediatric.nagasaki~~furuoyanoriko~~@gmail.com

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ABSTRACT

~~**Background:** Organophosphate pesticides (OPs) are widely used widely;: however, only a few epidemiological studies have investigated the association between neurologicalbehavioral or neuropsychological effects and occupational OP exposure.~~

Objective: The aim of this study was to conduct a systematic review of ~~the~~ published literature~~s~~ and to estimate whether or not there is a causal relationship between occupational exposure to Organophosphate pesticides (OPs) and either neurologic impairment or depressive symptoms.

Data sources: EMBASE, MEDLINE, Global Health, and PsycINFO (1980 to April 2014).

Setting: Observational studies (cross-sectional, cohort, and case-control studies) with both exposed and unexposed groups.

Participants: People who occupationally use OPs more than one month and their family.

Primary outcome: Results of neurological core test batteries or depressive symptoms such as headaches, anxiety, and dizziness.

Study appraisal and synthesis methods~~**Method**~~: After ~~A~~an extensive search of various literature databases, one author screened titles and abstracts, searched the relevant publications manually, and conducted data extraction. ~~was conducted, and the relevant publications were then manually searched manually.~~ All ~~the relevant data wereas~~ extracted data from the selected articles were~~and~~ synthesized for analysis. Quality appraisal was conducted using Newcastle Ottawa Scale. ~~Meta-analysis was implemented using mean scores of the neurologic tests and depressive symptoms.~~

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Results: Of the 1024 articles retrieved by database search, 24~~Twenty three~~ studies that met inclusion and exclusion criteria were selected for analysis. Of the selected studies, 17~~6~~ were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10~~nine~~ studies), UK (four studies), Africa (four studies), Asia (three studies), Europe (two studies), and ~~one in~~ South America (one study). E~~The E~~ach of the included studies~~y~~ used different exposure and outcome assessments such as neurologic scores and depressive symptoms, ~~thus~~ making it difficult to compare the results exactly. ~~The m~~Most studies showed that ~~the~~ exposed groups had poorer results than ~~the~~ unexposed groups; ~~;~~ however, because of inconsistent neurological test batteries there was not enough pooling evidence to conduct a meta-analysis~~evidence based on the results of the meta-analysis was weak~~.

Conclusion: The findings of this literature review indicate that ~~there~~it is a necessary~~necessity~~ to standardize the neurological~~behavioral~~ or neuropsychological test battery and methods of measuring ~~OPs~~ exposure to OPs.

Trial registration: Not applicable.

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~~there might be a causal relationship between occupational exposure to OPs and neurological impairment or depressive symptoms.~~

ARTICLE SUMMARY

Article Focus

- To systematically review epidemiological studies that~~which~~ examine adverse effects on the human central nervous system (CNS) by exposure to organophosphate pesticides (OPs).

Key messages

- OPs have been ~~widely~~ used widely all over the world for agricultural or industrial use.
- Many ~~There are a plenty of~~ studies have~~which have~~ examined acute health problems caused by OPs~~:~~, however, few studies have investigated negative effects caused by occupational OPs exposure.

Strengths and limitations of this study

- The article represents a systematic review of epidemiological studies on adverse effects on the human ~~central nervous system~~CNS by occupational OPs exposure, with a quality appraisal of each study.
- The article identifies problematic issues of exposure and outcome assessments.
- M~~The m~~eta-analysis ~~was limited because each study used various outcome assessments~~could not be applied ~~due to~~because only a small number of ~~the~~ pooled studies were available.~

- In some studies~~,~~ ~~There~~ it ~~is~~was ~~a~~ difficult~~y~~ to judge negative effects caused ~~by~~ only by OPs, because mixed pesticides were used ~~in some studies~~.

INTRODUCTION

Ever since organophosphate pesticides (OPs) were developed, they have been used tofor combating insects for public health purposes and to support agricultural productivity and manufacturing processes. SinceBecause Ppesticides are also well-known as one of the leading suicide methods, and approximately three million cases of pesticide poisoning occur every year around the world. This is especially prevalent in Asian nations including Sri Lanka, China, and Malaysia (1). For this reason, a large number of epidemiological studies have investigated the relationship between high level OPs exposure such as pesticide poisoning and accidents and acute health effects(1, 2). and It has been reported that high level OPs exposure is significantly related to neurological or neuropsychological impairment (1, 2)((1, 2)2, 3). In contrast, few studies that have reportinvestigated associations between occupational or cumulative OPs exposure and negative effects on human health are available, even although. Although some research has examined the negative influence onto young children ofby cumulative OPs exposure (3, 4)((3, 4)4, 5) andor others have investigated relationships between reproductive health and occupational OPs exposure (5-7)((5-7)6-8).. Since hHigh levels OPs exposure providesare known to have adverse effects on the human CNScentral nervous system, therefore, occupational or cumulative OPs exposure has also has the potential to negatively affect the CNSit. However, there are very few epidemiological studies thatwhich have assessed the relationships between occupational OPs exposure and neurologic or mental problems have been published using epidemiological research. The objective of this systematic review is to verify

whether or not occupational OPs exposure ~~could~~ negatively affects ~~influence on~~ the human ~~central nervous system~~CNS. ~~In this systematic review~~To investigate this further ~~test the hypothesis~~, we summarized the epidemiological evidence for the relationship between occupational OPs exposure and mental and neuropsychological aggression, especially for occupational OP users, ~~is summarized,~~ and ~~and~~ some of the limitations associated with the various studies are discussed.

MATERIALS AND METHODS

Searching strategy for identification of published studies

~~A~~We search~~ed the published literature for observational studies was carried out~~ using the Ovid SP~~(8),~~ ~~a~~ search software (8) to select relevant observational studies~~, by the author~~. A ~~G~~geographical ~~and time~~ restriction~~s~~ ~~were~~was not imposed~~;~~, however, the search~~a published period~~ was restricted to studies published from 1980 to 2014~~Current~~. Population-based case-control studies were excluded from the systematic review because it was~~is~~ difficult to assess accurate exposure doses for these studies. Because ~~Currently,~~ various pesticides including OPs~~, currently,~~ are currently easily ~~-~~available to~~for~~ everyone, ~~and~~ ~~some people have a~~it is ~~possibility~~highly likely that ~~of using~~these pesticides have been obtained for personal use. ~~However~~For this reason, it is almost impossible to ~~comprehend exactly~~obtain past records of pesticide~~s~~ use by every ~~person~~individual. The literature search was limited to studies in humans and to reports published in English, and the review was limited to

epidemiological studies. Moreover, unpublished studies and grey literature (literature that has not been formally published)s were not searched in this systematic review;, therefore we did not make a contact with any authors to find out unpublished studies. Studies investigating OPs exposure through food and water contamination were also excluded. A search of the following four databases was carried out:

1. EMBASE Classic +plus EMBASE (198047 to 201Week13 20140 July 09);,
2. Ovid MEDLINE(R) (19850 to June Week 5 2010March Week134 2014);,
3. Global Health (198010 to June 2010Week12 2014);, and
4. PsycINFO (1980806 to July Week 1 2010April Week14 2014).

A combination of free-text terms and explore terms was used to identify relevant articles. For exposure, the following search keywords were used: organophosphate*, organophosphorous, pesticide*;, or or insecticide*, and organophosphate pesticide (explore map term);. pesticide (explore map term). For outcome, the following search keywords were used: neuro*, psychiatr*, psycholog*, mental health, mental illness, mental disorder, or depressi*, depression (Epidemiology) (explore map term), and mental health (explore map term). For subjects, the following search keywords were used: occupation*, agricultu*, or farm*. For study design, the following search keywords were used: epidemiolog*, cohort, or cross-sectional, or case-control, andor Eepidemiology (explore map term) were used as keywords. An initial systematic search in the titles and abstracts was conducted using a combination of all these search terms. A second manual search of the

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reference lists from the selected relevant articles was performed to explore or retrieve articles found in the initial search in order to find ~~out~~as many available studies ~~to the extent~~as possible.

Criteria for selecting studies for ~~the~~ review

Only original research articles meeting the inclusion and exclusion criteria described below were used in the final review~~result~~.

Inclusion criteria:

1. ~~–~~Study design~~:~~

a) Must be observational studies: cross-sectional, cohort, and case-control studies.

b) Studies must have both exposed and unexposed groups.

2. ~~–~~Subjects~~:~~

a) The subjects in the exposed group either must use OPs occupationally, or there must be a probability of being exposed to OPs during their work.

b) The families of occupational OP users can be treated as subjects.

3. ~~–~~Exposure~~–~~

a) Subjects must be exposed to OPs for at least one month.

b) Seasonal workers who used OPs for more than one month must be included.

4. ~~–~~Outcome~~–~~

Studies must have carried out some tests to assess damage toof the CNS ~~(Central Nervous System)~~ or have conducted a survey or an interview to identify depressive symptoms.

5. –Exposure-outcome association

Results must be reported as some types of relative risks or mean scores.

Exclusion criteria:

1. Study design

Experimental and laboratory based studies including animal studies were excluded. Population-based case-control studies were excluded.

2. Subjects

Studies of mainly patients of pesticide poisoning were ~~not~~ excluded.

3. Exposure

Studies thatwhich did not specify the type of pesticides were excluded.

4. Outcome

Studies examining damage of the peripheral nervous system due to OPs exposure were excluded.

5. Language

Studies published in a language other than English were excluded.

Definitions used for the review

Definition of cumulative exposure

a) People who used OPs in their jobs for at least one month and had~~ve~~ the~~a~~ probability of inhaling ambient OPs and absorbing OPs by spraying and touching.

b) Families of OP users were included as subjects~~,~~ because they may have been exposed to OPs by washing clothes contaminated by OPs and/or by touching OP users.

Definition of poor mental health

A) Neurological or neuropsychological impairment

a) People who had poorer results in neurological or neuropsychological test batteries than healthy people of the same age.

b) People who had short-memory loss~~;~~ for example, people who had experienced memory loss of six to three months duration.

B) Depressive Symptom

c) People who, regardless of their age, had chronic depressive symptoms including headache, fatigue, dizziness, sleepless, and eye problems.

d) People who were diagnosed with depression by clinical doctors.

Study selection process

Using the search terms listed above, a total of 1024~~592~~ references were obtained: 515~~276~~ from EMBASE~~mbase~~ Classic + EMBASE~~mbase~~, 31~~16~~ from PsycINFO, 196~~133~~ from Global Health, and

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282~~167~~ from Ovid MEDLINE(R)~~edline~~ (Figure1). However, 77 animal studies, ~~were excluded because they were not appropriate to test the hypothesis of this review. Furtheremore,~~ 90 studies ~~were~~ not in English studies, and 12 studies that did not meet ~~were removed due to~~ the time restrictions were excluded. Of the remaining 845 studies, 516~~197~~ ~~of 845592 references~~ were excluded ~~due to~~because of duplications~~es~~. ~~Of these 395 unique references that remained, 63 were not in English, and 32 were animal studies.~~ A manual search of the titles and abstracts of the remaining 32900 references excluded a further 272~~268~~ studies. The 21~~32~~ remaining articles were fully reviewed, after which 13~~2~~ studies were deemed to meet inclusion and exclusion criteria (9-20) ~~(8-20)((8-20)9-21)~~. In addition, 12~~0~~ articles identified by the manual search were added to the systematic review (Figure1~~See Appendix A for flow of study inclusion and exclusion diagram~~). To include as many relevant studies as possible, studies published before 1980 that were found by the manual search were included to the list for review. Finally, these 24~~3~~ studies were ~~identified~~selected ~~and used~~ for data extraction (9-32)~~(21)(8-31). ((8, 11, 13, 15, 19, 21-38)22-31).~~

Data extraction, synthesis, and analysis

Data extraction forms were created to compare relevant data collected from each of the 24~~3~~ studies. The following data were~~as~~ extracted to assess heterogeneity of the included studies~~as basic data~~:~~Extracted data included~~ title, authors, year published, ~~and the~~ number of subjects in the exposed and unexposed groups, occupation, and demographic information such as mean age, sex,

smoking status, and geographical area~~,~~. In addition ~~to basic data~~, the following data were~~as~~ extracted to assess confounding factors and statistical models among the included studies: inclusion and exclusion criteria such as first language, alcohol consumption, ~~and~~ injury experience, confounding factors, and statistical methods used.~~,~~ The following data were~~as~~ extracted to assess exposure and outcomes ~~assessment~~: types of pesticides, exposure assessment, and ~~statistical methods,~~ outcome assessment to measure the neurologic or neuropsychological ability, and results obtained. Tables containing the data that were~~as~~ obtained using the data extraction forms were constructed~~–~~ and analyzed~~.~~ –P-values and 95% ~~percent~~ confidence intervals (95%CIs) were elicited from the articles to judge statistical uncertainty. When a study had investigated depressive symptoms, the information was collected and a table was constructed. Impact and statistical magnitude of depressive symptoms were represented using plus or minus signs ~~including~~ '++', '+', and '–', based on the P-value or 95%CI of the studies. ~~Meta-analysis was carried out using mean scores of neuropsychological tests with STATA version 11.0.~~ All data extraction, coding, and quality appraisal were~~as~~ conducted only~~by only~~ by the first author~~;~~ therefore, no~~events in~~ disagreement events ~~were not~~ occurred.

Quality appraisal

The quality of the 24~~3~~ studies was appraised using a scale ~~that was~~ adapted from the 'Newcastle/Ottawa Scale (NOS)'(33) ~~(32)~~ (The appraisal standard of NOS is~~was~~ shown in Appendix A~~B~~). Based on the NOS, each study was evaluated using the point system. When a study included

relevant information that could be associated to the NOS, one point was added. FThere are five items in cross-sectional studies and eight items in cohort and case–control studies that couldcan be related to the NOS were identified. Therefore, cross-sectional studies assigned 5, 4, 3, or 0–2 points were evaluated as very good, good, satisfactory, or unsatisfactory studies, respectively. Similarly, cohort/case-control studies with 7–8, 5–6, 4, and 0–3 points were identified as very good, good, satisfactory, or unsatisfactory, respectively.

RESULTTLS

As a result of the search strategy described in the Materials and Methods section, 123 studies were identified from the database search and another 120 studies were found after a manual search. A total ofThese 243 articles, published between 1975 and 20100, met all the inclusion and exclusion criteria. A summary of the characteristics of the 243 selected articles is shown in Table 1.

Study design and geographical area–

Of the selected studies, 176 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10nine studies), UK (four studies), Africa (four studies; two in South Africa, one in Egypt, and one in Kenya), Asia (three studies; two in India, and one in Sri Lanka), Europe (two studies; one in Spain and one in Poland), and one in South America (one study; Ecuador, one study).

Characteristics of subjects

Because the subjects were limited to people who had the probability of being occupationally exposed by OPs, the majority of the participants (60−70%) were men. Most of the time, agricultural work such as pesticide application and farming is ~~has been~~~~is predominantly~~ performed predominantly by men. Six~~Five~~ ~~out~~ of the 24~~3~~ studies included both male and female subjects~~,~~ ~~however, approximately 60 to 70 percent of the subjects were male~~ ~~(~~(9, 11, 17, 25, 27, 32)~~9, 21, 27, 29, 33)~~, and~~,~~ o~~O~~nly one study used all female subjects in both the exposed and control groups (21)~~(23)~~. In 13~~2~~ of the studies ~~T~~the mean age of the exposed subjects was in the ~~thirtie~~30s ~~in 12 studies~~, in six studies the mean age was in the 40~~forties~~ ~~(~~(9, 14, 15, 19, 20, 31)~~,9, 12, 16, 17, 24, 34)~~ and in two studies the mean age was in the ~~fiftie~~50s ~~(~~(13, 17)~~13, 21)~~. The mean age in two~~one~~ studies~~y~~ was ~~in the twenties, however, the mean age was~~ 29, very close to thirty ~~(~~(25, 32)~~27)~~. One of the studies~~y~~ did not report detailed demographic data of the participants ~~(10)~~(10).

Source of recruitment and sample size

Ten out of the 24~~3~~ studies were on pesticide applicators including private, commercial, and tree, fruit, and vegetable applicators. Five~~our~~ and three studies were on farmers and sheep farmers, respectively, and~~,~~ two studies were on factory workers and greenhouse workers. One study investigated depressive symptoms in the spouses of OPs users. In the study by Korsak et al. (22), the

specific occupation of the population in the study was not stated, however, the subjects had experienced occupational OPs exposure~~ (25(21))~~. The number of subjects in the exposed groups varied from 16 to 2,051, while the control groups had a wider range of subjects~~, with the figure ranging from~~ (16 to 27,023).

Table1 ~~Reported~~ Ffindings reported in ~~recent~~ epidemiological studies ~~regarding~~into occupational low level OPs exposure and mental illness

	Author	Study Design	Country	Exposed Population(No)	Chemical	Exposed Assessment	Comparison Group
1	Albers et al (9)~~(9)~~	CO	USA	Chemical workers(53)	OP	Industrial HR,AChE INH	Similar workers, not exposed(60)
2	Bazylewicz-Walczak et al (21)~~(23)~~	CO	Poland	Greenhouse workers(26)	OP	DR	Greenhouse workers, not exposed(25)
3	Beseler et al (10)~~(10)~~*	NC/ CO	USA	Case**: Spouses of private applicators with depressive diagnoses(2,051)	OP	QU or IN	Control: Spouses of private applicators without depressive diagnoses (27,023)
4	Cole et al (11)~~(33)~~	CR	Ecuador	Farmers, some applicators(144)	OP,CAR, FNG	IN, QU, AChE INH	Local Population(72)
5	Daniell et al (12)~~(20)~~	CO	USA	Farm worker applicators(49)	OP	QU, AChE INH	Slaughterhouse workers(40)
6	Dassanayake et al (13)~~(13)~~	CR	Sri Lanka	Vegetable farm workers (38)	OP	N.A.	hospital labours(35)
7	Farahat et al (14)~~(24)~~	CR	Egypt	Farm workers(52)	OP	AChE INH	Local Population(50)
8	Fiedler et al (15)~~(34)~~	CR	USA	Tree fruit farmers (57)	OP	QU, lifetime exposure metric	Cranbury/blueberry growers(low exposed), hardware storeowners(unexposed) (42)
9	Korsak et al (22)~~(25)~~	CR	USA	Occupational exposure(16)	OP, CAR, OC	AChE INH	Local Population(low exposure)(16)
10	Levin et al (23)~~(26)~~*	CR	USA	Pesticide applicators(24)	OP	IN, AChE INH	Farmers(24)
11	London et al (16)~~(18)~~	CR	South Africa	Fruit farm pesticide applicators(163)	OP	QU(job-matrix)	Farm workers, not applicators(84)
12	London et	CR	South Africa	Fruit farm pesticide applicators(164)	OP	QU (job-matrix)	Farm workers, not applicators(83)

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	al(24)~~(15)~~*						
13	Maizlish et al (25)~~(27)~~	CR	USA	Pesticide applicators(46)	OP	UM, DR	Non-applicators(56)
14	Misra et al (26)~~5(28)~~*	PR	India	Pesticide applicators(22)	OP	AChE INH	Hospital labours(20)
15	Ohayo-Mitoko et al (27)~~(29)~~*	CO	Kenya	Farm worker applicators(256)	OP, CAR	AChE INH	Farm workers(low exposure)(152)
16	Rodnitzky et al (28)~~(30)~~	CR	USA	Pesticide applicators(23)	OP	AChE INH	Farmers(23)
17	Roldan-Tapia et al (18)~~(14)~~	CR	Spain	Greenhouse workers(40)	OP, CAR	QU, AChE INH	Local Population(26)
18	Ross et al (17)~~(21)~~	CO	UK	Sheep farmers(127)	OP	IN	Police workers(78)
19	Rothlein et al(32)	CR	USA	Farm workers(96)	OP	UM, House dust	Workers in hotels and tourist industry(45)
20 ~~19~~	Srivastava et al(29)~~(31)~~	CR	India	Manufacture workers(59)	OP	AChE INH	Manufacture workers, not exposed(17)
21 ~~20~~	Steenland et al(30)~~(11)~~	CR	USA	Termiticide applicators(191)	OP	IN,UM	Friends, blue collar workers(189)
22 ~~1~~	Stephens et al (19)~~(12)~~	CR	UK	Sheep farmers(146)	OP	QU	Quarry workers(143)
23 ~~2~~	Stephens et al (31)~~(17)~~	CR	UK	Sheep farmers(77)	OP	QU, UM	Quarry workers(69)
24 ~~3~~	Stephens et al (20)~~(16)~~	CR	UK	Orchard applicators(37)	OP	IN,QU	Construction workers,pig farmers(57)

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Study Design CR: Cross-sectional, CO: Cohort, NC: Nested case-control, PR: Prospective study

Chemical OP: Organophosphates, OC: Organochlorines, CAR: Carbamates, FUN: Fungicides, AChE: Acetylcholinesterase

Exposed Assessment : AChE INH: AChE inhibition, DR: Dermal and Respiratory Absorption, IN: Interview , QU: Questionnaire , HR: Hygiene Records UM: Urinary metabolites.

*ArticlesStudies that including ed depressive symptoms for outcome assessments.

**Cases were defined as female spouses of private applicators who responded 'yes' to the question "Has a DOCTOR ever told you that you had been diagnosed with depression requiring medication?" Controls were female spouses who responded 'no' (10).

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Exposure assessment

Exposure assessment in the included studies could bewas divided, for the most part, into five patterns: indirect assessment using, for example, an interview or questionnaire; direct assessment including athe measurement of urinary metabolites and acetylcholinesterase (AChE) levels in the blood or a measurement of ambient OPs using a patch and a pump; a combination of direct and indirect methods; a combination of a biomarker and OPs exposure levels included in house dust;; and a combination of biomarkers and ambient OP levels. Seven out of the 243 studies used indirect methods, and six studies used blood AChE inhibition levels to measure AChE levels in the blood as an exposure indicator. Sixix studies used a combination of indirect methods and biomarkers, and three studies used biomarkers and the ambient OP levels, one study used a biomarker and house dust. The remaining study did not mention any exposure assessment methods. In all the studies thatwhich used urinary metabolites as exposure assessment, the results were presented as the sum of dialkylphosphates (DAP) (i.e. the sum of six DAP metabolites: DMP (dimethylphosphate), DMTP (dimethylthiophosphate), DMDTP (dimethyldithiophosphate), DEP (diethylphosphate), DETP (diethylthiophosphate), and DEDTP (diethyldithiophosphate)) (25, 30-32)(8).

Outcome measurements

Two different outcome measurements were used in the studies; one measured neurological impairment and the other assessed depressive symptoms. Of the 243 studies, 198 used cognitive function tests to investigate negative neurologic influencess caused by OPs exposure.

Associations between outcome and exposure

Ten of the 198 studies that investiginged cognitive impairment mentioned that at least one measure outcome showed more impairment in the exposed groups;, however, these observations were not significant (P-<0.05). Seveni~~x~~ of the studies reported some significant positive associations of exposure with poor outcome (P-<0.05);, however, even in these cases, the significant decrements were observed only in some of the neurologic tests, mainly in the Digit Span and Santa Ana Dexterity tests. Indeed, there are several versions of these neurologic tests and the significance of the scores often depended on the versions of the tests that were used. ~~Five studies used the Neurobehavioral Evaluation System (NES), f~~Fiveour studies used the Wechsler Adult Intelligence Scale (WAIS or WAIS-R) (34, 35), four studies used the Neurobehavioral Evaluation System (NES) (36)~~(35),~~ two studies used the World Health Organization

Neurobehavioral Core Test Battery (NCTB) (37, 38)~~(37)~~, and the remaining eight ~~four~~ studies used their own scales.

Five studies adopted depressive symptoms as outcome measurements, as shown in ~~the~~ Table2;~~,~~ however, the symptoms used in the studies were not standardized.

Table~~-~~2 ~~The~~ Summary ~~table~~ of depressive symptoms used as outcomemeasurements

Reference	~~Obtained~~ Results obtained	Impact of outcomes
Beseler et al 2006(10)~~(10)~~	Depression due to doctor's diagnosis was not significantly related to low (OR 1.09; 95%CI 0.91, 1.31) or high (OR 1.09; 95%CI 0.91, 1.31) cumulative exposure.	-
Levin et al 1976(23)~~(26)~~	Anxiety score of the pesticide applicators was significantly higher (P<0.05) than that of the farmers. However, there was no significant difference in measures of depression.	++
London et al 1998(24)~~(15)~~	Dizziness, sleepiness, and headache ~~–~~had a significantly higher overall neurological symptom score (P<0.05).	++
Misra et al 1985(26)~~(28)~~	Common symptoms were Headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%) and no neurologic change was seen.	-
Ohayo-Mitoko et al 2000(27)~~(29)~~	A significant change in symptom prevalence was found for the respiratory (2.48% CI (0.78, 5.38) and central nervous system (2.56% CI (0.99, 6.62), but in terms of skin~~,~~ symptoms~~stematic~~, and eye symptoms, there was no statistically significant change.	++

OR=Odds Ratio ++: Statistically significant (P~~p~~<0.05), -: Not statistically significant

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Statistical analysis

Sixteen studies used logistic regression, and the remaining eight~~seven~~ used other

statistical tests including X^2-test and t-test. Only one study adjusted for sex in the logistic regression. Fourteen~~Thirteen~~ ~~out~~ of the 2~~43~~ studies adjusted for age, and 1~~21~~ adjusted for education in the~~–~~ statistical ana~~l~~lysis~~logistic regression~~. However, only five studies adjusted for alcohol consumption before carrying out the statistical analysis, and ~~, Further,~~ only two studies adjusted for first language.

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Methodological quality appraisal

Based on NOS, ~~F~~five~~our out~~ of the 2~~43~~ studies were of very good quality, 10 were of good quality, and the remaining nine were either satisfactory or unsatisfactory. Most ~~of the bad quality~~ studies with unsatisfactory scores~~quality~~ either were carried out before 1990 or were performed in some of the less developed countries. In particular, the methods of recruitment of subjects, controlling for confounders, and outcome assessment were not appropriate. For example, in some~~ of the~~ studies, all of the participants were volunteers ~~(~~(14, 28)~~24, 30)~~ and in another study, the subjects were not representative of the community from which they were recruited (factory workers) (29)~~(31)~~. In addition, in the unsatisfactory studies, how the outcome was assessed was not described~~ in the unsatisfactory studies~~, and ~~some of the~~ methods needed to avoid confounders such as stratification and regression were not used. ~~On the other hand,~~

~~n~~None of the cohort studies were assessed as very good quality because most of them did not have a long enough follow-up duration (in five studies, the duration was less than six months) and the selected subjects were not fully representative of the target community. Moreover, the methods of outcome assessment were not described in most of the cohort studies.

Data synthesis ~~and meta-analysis~~

~~As shown in Figure 1 and 2, a meta-analysis was carried out using the reported mean scores for the implemented neurobehavioral test; however, because the investigators used different scoring systems, meta-analysis was difficult.~~ The results of the neurologic tests used in the studies are~~were~~ summarized in Table3. As can be seen ~~in the Table3~~, the test batteries differed from ~~each~~ study to study. The commonly used test~~s~~ batteries in NCTB, NES, and WAIS were Symbol-Digit and Digit Span Forward and Backward. However, some studies that adopted NES and WAIS to measure neurologic impairment ~~(Table3)~~ implemented only a few subsets in the trials. Among the 13~~five~~ studies that us~~ed~~ing a Symbol-Digit test, ~~each~~ four~~three~~ used NES and u~~U~~nknown tests, ~~each~~ ~~WAIS,~~ two used WAIS and WAIS-R ~~and unknown tests~~, and one ~~was~~used a Polish NCTB. ~~For~~Among the studies that used Digit Span~~, there were f~~Forward and

bBackward tests, some studies performed both tests, butwhile the others did eitheronly one of the tests as shown in Table3 test, two studies used NES and WAIS in the forward tests and two WAIS in the backward tests. Overall As a result, there were only four of the studies that used the same test battery in NES and WAIS , respectively, and it was impossible to perform a meta-analysis for neurological test batteries. Because there were only two studies in each Digit Span test, a meta-analysis would not be very useful, and so a meta-analysis for the Digit Span tests was not carried out and only a meta-analysis for NES and WAIS Symbol Digit tests was performed. In terms of Symbol Digit (NES), slight positive association can be seen (Figure 1), while Figure 2 showed that there was no difference in mean score of Symbol Digit WAIS between the exposed and control groups. Although the three studies apparently used the same scoring systems, one of the scores was completely different from the scores in the other two studies. For example, the scores in the study byof Stephens et al. (31) were 24.22 and 21.01 in the exposed and the control groups, respectively (30)(17), whereas the scores reported byof Daniell et al. and Stephens et al. were much lower and between 2.23 and 3.55 (12, 20, 31)(16, 17, 20). Similarly, the mean scores reported by Bazylewicz-Walczak et al. (215) were higher, 45.50 and 49.40, while the mean scores reported in the other studies were smaller, 2.28 and 2.23 in the WAIS (25) (24)(27). In

consideration of insufficient number of studies and possible systematic differences in the population characteristics and/or in the measurement procedures between the studies, we decided not to conduct a meta-analysis.

Table 3 ~~The s~~Summary ~~table~~ of the neurologic test batteries used in some of the studies~~battery tests~~

Reference	Types of neurologic tests	Symbol Digit	Digit Span	Santa Ana	Simple Reaction Time	Syntactic Reasoning(s)
Bazylewicz-Walczak et al 1999(21)~~(23)~~	Polish NCTB/WAIS (Symbol Degit)	nd	nd	nd	**~~+~~	nd
Cole et al 1997(11)~~(33)~~	NCTB	nm	nm	nm	nd	nd
Daniell et al 1992(12)~~(20)~~	NES	*~~-~~	nd	nd	nd	nd
Farahat et al 2003(14)~~(24)~~	Unknown	***~~++~~	***~~++~~(f)[1]~~*~~ ***~~++~~(b)[2]**	nd	nd	nd
Fiedler et al 1997(15)~~(34)~~	WAIS-R	*~~-~~	*~~-~~	nd	***~~++~~	nd
London et al 1997(16)~~(18)~~	WAIS-R	nm	nm	***~~++~~	nm	nd
Maizlish et al 1987(25)~~(27)~~	WAIS	~~1/~~***~~++~~	nd	nd	nd	nd
Roldan-Tapia et al 2005(18)~~(14)~~	WAIS	***~~++~~ [3]~~*~~	***~~++~~ [3]~~*~~	nd	nd	nd
Ross et al 2010(17)~~(16)~~	WAIS	nd	***~~++~~	nd	nd	nd
Rothlein et al 2006(32)	Unknown	*	*(f)[1] ***(b)[2]	nd	*	nd
Srivastava et al 2000(29)~~(31)~~	Unknown	***~~++~~	***~~++~~	nd	nd	nd
Steenland et al 2000(30)~~(11)~~	NES	*~~-~~	*~~-~~	nd	*~~-~~	nd
Stephens et al	Unknown	***~~++~~	*~~-~~	nd	***~~++~~	**~~+~~

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1995(19)(12)						
Stephens et al	NES/ACT	nm	nm	nd	nm	nm
1996(31)(17)						
Stephens et al	NES/ACT	*-	*-	nd	*-	***++
2004(20)(16)						(ACTS)

***++: P<0.05, **+: 0.05≦P<0.1, *—: P>0.1;

The eExposed groups were slower or had poorer outcomes than the control groups

[1]1: (f) Digit Span fForward

[2]2: (b) Digit Span bBackward

[3]†: The article did not mention wWhether the obtained results were positive or negative was not reported in the studyies.

nd: The sSubsets of neurological tests were not performed.

nm: Although the sSubsets of neurological tests were performed but P-values were not mentioned in the articlereported.

*(f) Digit Span forward, **(b) Digit Span backward

DISCUSSION

The systematic keyword and manual searchesresults showed that there were of the published literature identified 243 epidemiological studies thatwhich examined the relationship between OPs and CNS by systematically searching. When the relevant information was assessedcomparing the selected studies by each item, two main findings were obtained,; one wasis the method of exposure assessment, and the other wasis the method used for the outcome measurement. With respect toFor exposure assessment, the matter of measurement methods wereas categorized asinto three: direct, indirect, and a combination of both methodsdirect and indirect. For the On the other hand, in terms of outcome measurements, there seemed to be two main

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assessments~~ways~~ were used,~~to gauge~~ neurologic impairment and depressive symptoms.

Exposure assessment

Exposure assessment was not used for group allocation in all the studies;~~,~~ ~~and~~rather, it was implemented to measure how much subjects were exposed and the outcomes of the neurobehavioral tests. D~~Each study used d~~ifferent exposure assessment methods were used in each study, which made it difficult to accurately compare the studies. In addition, there seemed to be methodological imperfections in both the direct and indirect methods. For example~~To illustrate~~, in one study, an interview~~s~~ and questionnaire~~s~~ were used ~~in the indirect method, though, one study~~for ~~the~~ recruited subjects over 60 years old who had been retired for 11 years ~~since their retirement~~ (17)~~(21)~~. This method is subject~~has the potential of causing~~~~In this study,~~ to recall bias ~~could be a problem~~ because the rate of cognitive impairment is likely to have increased as the subjects aged ~~put on years~~. ~~This could lead to inaccuracy of exposure assessment~~. However, other indirect methods, especially extensive history records of pesticide use could be considered as a proxy of how much OPs might have accumulated in the body, thus records of this type can be used to estimate the amount of OPs by long-term exposure, even though there may be some recall bias. ~~With respect to~~For the direct method~~s~~, ~~there were several ways to~~

detect OPs. Although some studies used DPAbiomarkers useor dsuch as urinary metabolites as an indicator of exposure was used, as an exposure index in the study; however, DPA is metabolized rapidly and excreted from bodies (6)(7). Therefore, measuring urinary analysis was not a perfect way to assess OPs exposure, oOn the contrary, it seemed that measuring AChE levels was the most reliable way to assess the amount of OP exposure, because the blood AChEcholinesterase levels needstake approximately one week to becomes normal by being synthesized into a new molecular of AChE, which takes around a week (39)(35). hHence, althoughthe amount of OP exposure within one week can be accurately measured by AChE inhibition level in blood, but the blood AChE levelsthis cannot be be used to assessed the amountaccumulation of of OPs exposure accumulated in body tissues over for a long time, it Thus, direct method using the levels of AChE in blood is appropriate forcan be used to assessing short-term exposure, however, it is not for long-term exposure. On the contrary, iIndirect methods, especially extensive history records of pesticides such as structured interview and questionnaire could be a proxy helpful to grasp the past information about OPs usehow much OPs were accumulated in the body, even though there may be some recall bias. In order tTo minimize measurement errors, a mixed method for the assessment of short-term and long-term exposure should be established.

~~a it is desired that standardized measurement method should be established for further research. a combination of direct and indirect methods should be used.~~

Outcome assessment

The main problem in analyzing the outcome measurements was the inconsistencies in ~~the results of~~ neurologic test batteries~~ were not consistent~~~~differed from each study~~. V~~,~~ ~~and even if the same test battery was used, the types of tests such as NES and WAIS were different.~~ ~~To elaborate, as shown in Table 3, three studies adopted WAIS and four used NES as outcome assessment, and since there were v~~various versions of the neurologic tests were used in the studies and ~~battery tests including WAIS and WAIS-R,~~ the content of the tests ~~slightly~~ differ slightly ~~from~~in each study (Table3). Therefore, only a few tests were common across some of the studies, which made it difficult to compare the studies. Further, a meta-analysis could not be applied because of the insufficient ~~a small number of~~ number of studies. M~~Performing a m~~eta-analysis could have been performed ~~might be possible~~ by dividing the results into subgroups~~,~~; however, the ~~A meta-analysis using~~ results ~~that would be obtained from the meta-analysis~~ could

be highly misleading due tobecause of loss of power of studies (40) and cause sampling and publication biases. a small number of studies has the potential of causing sampling and publication bias due to small effect size, and even if a meta-analysis was implemented, the reliability would be low. Similarly, iIn terms of depressive symptoms, the outcomes assessment was againere different infrom each study. , fFor instance, one study usedhad the proportion of headaches, while the another used that of dizziness and sleepiness as the main outcomes. To gain better insights into whether precise conclusion that occupationalcumulative OP exposure can negatively affect the human CNS or not, at the very least, avoid these problems, aneurologic test batteriesbattery tests, at least, should be standardized outcome measurement and integrateda guidelines for measuring of neurologic symptomsimpairment should be set for all future epidemiological studies. As with exposure assessment, a similar problem can be seen in outcome assessment, for example, five out of the 23 studies adopted depressive symptoms as outcome measurements (Table 2). On the other hand, the remaining 18 studies used neurologic battery tests such as NES and WAIS. Thus the main problem in the outcome measurements is that comparison between the studies could not be done easily, because neurologic battery tests differed by each study. To elaborate, as shown in Table 3, three studies adopted WAIS and four used NES as outcome assessment, and since there were

~~various versions of neurologic battery tests including WAIS and WAIS-R, the content of the tests slightly differ from each study.~~ Furthermore, although some studies mentioned ~~about~~ the possible relationship between OPs exposure and confounding factors such as age and education, ~~they did not perform~~ statistical tests between the exposed and control groups were not performed in these studies. These inconsistencies ~~things obviously~~ make it difficult to compare the ~~outcomes of~~ neurologic impairment outcomes among the studies. ~~In addition, even in the same neurologic battery test, there are a variety of subtests such as Symbol Digit and Digit Span to measure neurologic impairment. The studies selected some subtests in their trials, hence there were few studies left to precisely compare. As a consequence, although the meta-analysis was carried out using the results of Symbol Digit, it was not enough to determine whether or not there was a statistically significant relationship. Similarly, in terms of depressive symptoms, outcomes were different from each study, for instance, one study had the proportion of headache, while the other used that of dizziness and sleepiness as main outcomes. Thus, neurologic battery tests, at least, should be standardized for further epidemiological research. If not, it could be difficult to gain precise conclusion that cumulative OP exposure can negatively affect human CNS or not.~~

Study design

Although 176 ~~Sixteen~~ of 243 ~~the~~ studies were cross-sectional studies, ~~and six were cohort studies.~~ ~~L~~longitudinal or cohort studies are more appropriate, ~~desirable rather than cross-sectional studies for three main reasons: one, in cross-sectional studies, it is difficult to confirm whether or not the disease preceded the exposure; two,~~ ~~B~~because ~~the~~ ~~outcome conditions in cross-sectional studies are too short lasting (36); and three, cross-sectional studies are suitable for investigating at a certain point, but they are not appropriate for mid-term studies. Especially,~~ agricultural work using pesticides is easily influenced by seasonality, ~~and o~~One research regarding reproductive health by OPs exposure stated that sperm concentration and counts are negatively affected ~~on~~in spring, peak season, ~~spring,~~ rather than winter (5)~~(6)~~. Therefore, ~~T~~the ~~result~~effect ~~on~~of the the CNS ~~neurobehavioral tests may~~could also be affected by seasonality; ~~therefore, cohort studies are ideal to assess the influence of occupational OPs exposure than cross-sectional.~~

Sources of ~~P~~possible biases

Only published studies written in English were searched, thus publication bias could have occurred. In future studies, non-English studies and unpublished studies should be

included to reduce publication bias. ~~If foreign workers are included i~~In ~~the~~ trials that included foreign workers, ~~their~~ first language and education levels ~~should~~could be considered as possible biases. ~~B~~ because there is a possibility that ~~the~~ non-native subjects ~~cannot~~did not fully understand the content and instructions ~~of~~for the tests, which could lead to them obtaining a lower score than ~~that of~~ native speakers. Additionally, the education systems in developed and less developed countries could be very different. Nowadays, developed countries such as USA and the ~~g~~Gulf countries have accepted foreign workers ~~from India and South American countries~~ as an important part of the work-force (12, 32, 41)~~(20, 37, 38)~~. These factors needed to be adjusted carefully in the sampling and analytical stages of the study; ~~H~~however, ~~in this systematic review, there were~~ only two of the selected studies ~~to~~ mentioned ~~about~~ first language in their statistical analyses ~~inclusion and exclusion criteria~~ (12, 31)~~(17, 20)~~. ~~OSince first language could influence the outcomes, it should be one of the factors to be considered when selecting subjects. Furthermore, when migrants and foreign labourers are included in the studies, education system is a point that we have to pay attention. Because education system between developed and less developed countries could be largely different. Hence, it is necessary to be careful when the results between subjects who come from different countries are compared. Additionally, o~~occupations could ~~be a~~

~~factor of~~also contribute to selection bias~~,~~ because, for example, a police officer or a~~and~~ construction worker~~s~~ would have a higher ~~possibility~~probability of experiencing ~~the~~ loss of consciousness due to accidents ~~of their jobs~~than workers with different occupations (17)~~ (21)~~.

Possible confounders

~~Age and social cultural factors are known as common confounding factors, though, not all studies adjusted them in the analysis. These factors could easily influence the results; hence they should be adjusted for further trials. Moreover,~~ Apart from common confounders such as age and education, ~~since~~ head injury and alcohol consumption could be other confounders, because~~have a probability of negatively affecting neurologic battery tests,~~ they can cause neurologic impairment due to memory deterioration.~~they should be treated as potential confounders as well.~~ Although some of the studies adjusted for alcohol consumption in the analysis (10, 12, 16, 24, 25)~~(10, 15, 18, 20, 27)~~, no study adjusted for head injury.~~However, the results showed that there was no study to adjust head injury in the logistic regression, on the other hand, there were some studies to adjust alcohol consumption in the analysis (10, 15, 18, 20, 27). Apart from these factors~~Furthermore, ~~participants'~~ nutrition status including vitamin

deficiency can~~is~~ also be relevant to the outcome of neuropsychological tests (16, 24)~~(15, 18)~~. Thus, ~~all~~ factors other than the common confounders that could negatively~~an~~ affect ~~measurements of~~ cognitive function should be adjusted for in the analysis.

~~Limitations~~Strengths and limitations of this review~~study~~~~weaknesses~~

~~Strengths~~

A major strength of this systematic review is that the characteristics of the selected studies were summarized using tables, and limitations of the exposure and outcome assessments used in these studies were ~~mainly~~ identified mainly on the basis of the constructed tables. Furthermore, the systematic review allowed us to propose recommendations that will be useful for standardizing future epidemiological research.

~~Weaknesses~~

All of the selected studies were relevant to occupational OPs exposure; however, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides that are commonly used in agriculture are usually mixtures of different pesticides, which are used to increase their effect. Four of the 2~~3~~ selected studies used a combination of OPs, organochlorines, carbamates, and fungicide; hence, the effect of only occupational OPs exposure could not be measured in these studies.

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InAlthough all of the studies which were collected in this systematic review were relevant to occupational OP exposure, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides usually are mixed with another type of pesticides to make their effects stronger, and this is the common in agriculture. In theis systematic review, four out of 23 studies were not single OPs exposure and they used a combination of OPs, OCs carbamates and fungicide,. which complicated Therefore, it may be quite difficult to measure the effect of only occupational OP exposure.

Of these studies, the outcome assessments18 assessed neurological or neuropsychological impairment using IQ tests. However, since the authors used the different neurological types of tests were usedbattery tests such as NCTB, NES, and WAIS, consequently, the lack of pooling evidence meant that there were only a few common tests including Digit Span and Symbol digit tests across the studies, comparisons among the studies became extremely difficult , furthermore, which made the comparison of the included studies more difficult. Hence, a meta-analysis wascould not be performed applied, to the two tests, but it is Small effect size due to a small number of studies may cause sampling and publication bias. and even if a meta-analysis is applied, it would be unreliable obvious that studies which can be appraised are

limited. In order to completely assess neurological impairment, there is necessity of the standardized tests battery for measuring neurological impairmentit is desirable that the same neurobehavioral test battery be used in a large number of studies. FurthermoreIn addition, the exclusionexcluding of studies written in languages other than English is one of theanother limitations of this studyreview.

CONCLUSION

AlthoughWhile some studies indicated negative influence on the human CNS based on the results of neurobehavioral or neuropsychological test batteries, the others did not. Hence, enough consistent results were not obtained to determine whether or not occupational OPs exposure could be harmful on the human CNS. the suggestive evidence for neurobehavioral test battery is inconsistent, there was a slight positive relationship of poor outcome implying that occupational exposure to OPs could be harmful for the CNS of the human. The evidence was weak in particular because some studies showed that there was a negative relationship of OPs with poor outcome. In addition, since tThe test items tested inof the neurologicalbehavioral or

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neuropsychological test batteries,~~y~~ and the estimates of OPs exposure were inconsistent because they depended on the preferences of the investigators~~, thus they were inconsistent.only a few items were common across the studies. Consequently, because there were only a few studies left, a meta-analysis could not be performed. for the meta-analysis; indeed, there were a few items which could be compared.~~ For future studies, the neurologicalbehavioral and~~or~~ neuropsychological test types, test batteries, and the methods used to measur~~ing method of OPsy~~ should be standardizsed ~~in order~~ to ensure adequate quality and to make it ~~more~~ possible to pool~~ing~~ the evidence from a large number of ~~the~~ studies for future analysis.

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FOOTNOTES

Contributors NT conceived the study design and participated in protocol development, literature searching, data extraction, data analysis, and drafted the manuscript. MH

critically reviewed the draft and contributed to the manuscript revisions.

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Competing interests None.

Ethical approval Systematic review.

Provenance and peer review Not commissioned, externally peer reviewed.

Data sharing statement No additional data are available.

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FIGURE LEGEND

Figure 1: Flow diagram of search and review process

~~Figure1 represents how the selected articles were searched. After electric search was conducted with restriction of published year, human, and English, a manual search of titles and abstracts was carried out. As a result, the remaining 21 studies were fully reviewed, and 12 studies met the inclusion and exclusion criteria. Another 12 studies were found by hand search.~~

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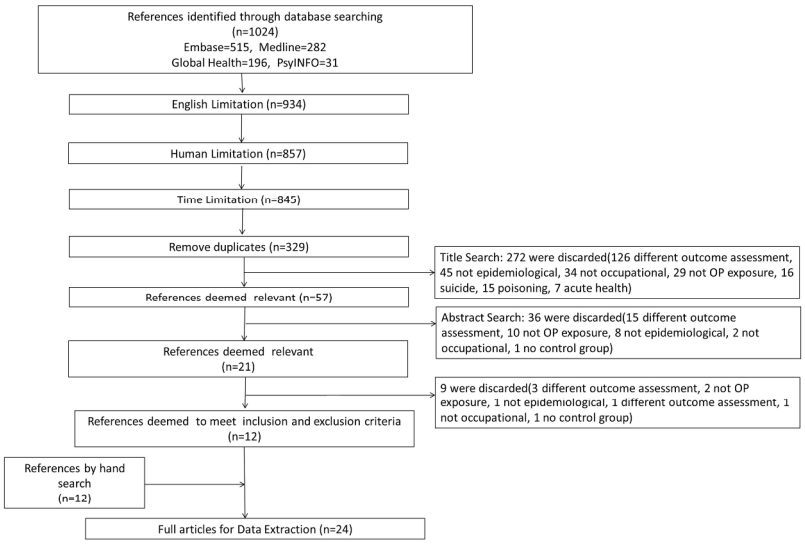

The figure1 represents the flow of database search and review process.
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Appendix A

The Appraisal Standard of Newcastle/Ottawa Scale

Selection

1) Representativeness of the exposed group/cohort

a) Truly representative of the average farmers or pesticides applicators in the community *

b) Somewhat representative of the average farmers or pesticides applicators in the community*

c) Selected group of users (e.g. factory workers, volunteers)

d) No description of the derivation of the group

2) Selection of the non-exposed group/cohort

a) Drawn from the same community as the exposed group*

b) Drawn from a different source

c) No description of the derivation of the non-exposed group

3) Ascertainment of exposure

a) Secure record (e.g. biomarkers)*

b) Structured interview or questionnaire*

c) Written self reports

d) No description

4) Demonstration that outcome of interest was not present at start of study *(Cohort Studies Only)*

a) Yes*

b) No

Confounder

1) Comparability of groups on the basis of the design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor* (e.g. alcohol consumption, smoking, and first language)

Outcome

1) Assessment of outcome

a) Independent blind assessment*

b) Record linkage*

c) Self reports

d) No description

2) Was follow-up long enough for outcomes to occur *(Cohort Studies Only)*

a) Yes (select an adequate follow up period for outcome of interest)*

b) No

3) Adequacy of follow up of cohorts *(Cohort Studies Only)*

a) Complete fellow up – all subjects accounted for*

b) Subjects lost to follow up unlikely to introduce bias – small number lost - > 70% follow up, or description provided of those lost*

c) Follow up < 70% and no description of those lost

d) No statement

Case Control Studies:

Selection

1) Is the case definition adequate?

a) Yes, with independent validation*

b) Yes, e.g. record linkage on self reports

c) No description

2) Representativeness of the cases

a) Consecutive or obviously representative series of cases*

b) Potential for selection biases or non stated

3) Selection of Controls

a) Community controls*

b) Hospital controls

c) No description

4) Definition of Controls

a) No history of disease (endpoint)*

b) No description of source

Confounder

1) Comparability of cases and controls on the basis of design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor*

Exposure

1) Ascertainment of exposure
a) Secure record (e.g. biomarkers)*
b) Structured interview where blind to case/control status*
c) Interview not blinded to case/ control status
d) Written self reports or medical record only
e) No description

2) Same method of ascertainment for cases and controls
a) Yes*
b) No

3) Non-Response rate
a) Same rate for both groups*
b) Non respondents described
c) Rate different and no designation
*: plus one point

There are five items in cross-sectional studies and eight items in cohort and case control studies, respectively. The quality of the studies was defined as follows.

Cross-sectional Studies:
Very Good Studies: 5 points
Good Studies: 4 points
Satisfactory Studies: 3 points
Unsatisfactory Studies: 0 to 2 points

Cohort / Case control Studies:
Very Good Studies: 7 to 8 points
Good Studies: 5 to 6 points
Satisfactory: 4 points
Unsatisfactory Studies: 0 to 3 points

Appendix B

Table1 Quality Appraisal (Cross-sectional Studies)

Selection	Cole et al 1997	Dassanayake et al 2009	Farahat et al 2003	Fiedler et al 1997	Korsak et al 1977	Levin et al 1976
1) Representativeness of the exposed group						
a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	a) (+1)	b) (+1)	c) (0)	a) (+1)	b) (+1)	b) (+1)
2) Selection of the non exposed group						
a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	b) (0)	b) (0)	a) (+1)	a) (+1)	b) (0)
3) Ascertainment of exposure						
a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	d) (0)	a) (+1)	b) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis	b) (+1)	- (0)	a) (+1)	- (0)	- (0)	- (0)
a) Study controls for age and						

education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)						
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	a) (+1)	b) (+1)	d) (0)	b) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	2/5 Unsatisfactory	2/5 Unsatisfactory	4/5 Good	3/5 Satisfactory	3/5 Satisfactory

Continued…

Table1 Continued

Selection	London et al 1997	London et al 1998	Maizish et al 1987	Rodnitzky et l 1975	Roldan-Tapia et al 2005
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	b) (+1)	a) (+1)	c) (0)	c) (0)	a) (+1)
2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	c) (0)	a) (+1)

3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	b) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	b) (+1)	b) (+1)	b) (+1)	- (0)	a) (+1)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	c) (0)	a) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	4/5 Good	4/5 Good	1/5 Unsatisfactory	5/5 Very Good

Continued…

Table1 Continued

Selection	Rothlein et al 2006	Srivastava et al 2000	Steenland et al 2000	Stephens et al 1995	Stephens et al 1996	Stephens et al 2004
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	b) (+1)	c) (0)	a) (+1)	a) (+1)	a) (+1)	a) (+1)

2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	a) (+1)	a) (+1)	c) (0)	a) (+1)	b) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	a) (+1)	- (0)	b) (+1)	b) (+1)	b) (+1)	- (0)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	d) (0)	a) (+1)	b) (+1)	d) (0)	b) (+1)
Overall Score	5/5 Very good	2/5 Unsatisfactory	5/5 Very Good	4/5 Good	4/5 Good	4/5 Good

Table2 Quality Appraisal (Cohort Studies)

Selection	Albers et al 2004	Bazylewicz-Walczak et al 1999	Daniell et al 1992	Ohayo-Mitoko et al 2000	Misra et al 1985	Ross et al 2010
1) Representativeness of the exposed cohort a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the cohort	c) (0)	c) (0)	a) (+1)	b) (+1)	c) (0)	a) (+1)
2) Selection of the non exposed cohort a)Drawn from the same community as the exposed cohort b)Drawn from a different source c) No description of the derivation of the non exposed cohort	b) (0)	a) (+1)	b) (0)	a) (+1)	b) (0)	b) (0)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	a) (+1)	a) (+1)	a) (+1)	b) (+1)	a) (+1)	b) (+1)
4)Demonstration that outcome of interest was not present at start of study a) Yes b) No	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption,	- (0)	a) (+1)	b) (+1)	- (0)	a) (+1)	a) (+1)

smoking, and first language)						

Continued…

Table2 Continued

Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	d) (0)	d) (0)	c) (0)	d) (0)	d) (0)
2) Was follow-up long enough for outcomes to occur a) Yes (select adequate follow up period for outcome of interest b) No	b) (0)	b) (0)	b) (0)	b) (0)	b) (0)	a) (+1)
3) Adequacy of follow up of cohorts a) Complete follow up-all subjects accounted for b) Subjects lost to follow up unlikely to introduce bias- small number lost- >70% follow up, or description provided of those lost c) Follow up rate<70% and no description of those lost d) No statement	b) (+1)	a) (+1)	a) (+1)	c) (0)	d) (0)	d) (0)
Overall Score	4/8 Satisfactory	5/8 Good	5/8 Good	4/8 Satisfactory	3/8 Unsatisfactory	5/8 Good

Table3 Quality Appraisal (Case-control Studies)

Selection	Beseler et al 2006
1) Is the case definition adequate?	
a) Yes, with independent validation b) Yes, e.g. record linkage or based on self reports C) No description	b) (0)
2) Representativeness of the cases	
a) Consecutive or obviously representative series of cases b) Potential for selection biases or not stated	a) (+1)
3) Selection of Controls	
a) Community controls b) Hospital controls C) No description	a) (+1)
4) Definition of Controls	
a) No history of disease (endpoint) b) No description of source	a) (+1)
Confounders 1) Comparability of cases and controls on the basis of design or analysis	
a) Study control for age and education b) Study controls for any additional factor	b) (+1)
Exposure 1) Ascertainment of exposure	
a) Secure record(biomarkers) b)Structured interview where blind to case/control status c) Interview not blinded to case/control status d) Written self report or medical record only	d) (0)

e) No description	

Continued…

Table3 Continued

2) Same method of ascertainment for cases and controls a) Yes b) No	a) Yes
3) Non-response rate a) Same rate for both groups b) Non respondents described c) Rate different and no designation	b) (0)
Overall Score	5/8 Good

MOOSE Checklist		
Section/Topic	**Checklist items**	**Check**
Title	• Identify the study as a meta-analysis (or systematic review)	X
Abstract	• Use the journal's structured format	X
Introduction (Present)	• The clinical problem	X
	• The hypothesis	X
	• A statement of objectives that includes the study population, the condition of interest, the exposure or intervention, and the outcome(s) considered	X
Sources (Describe)	• Qualifications of searchers (eg, librarians and investigators)	X
	• Search strategy, including time period included in the synthesis and keywords	X
	• Effort to include all available studies, including contact with authors	X
	• Databases and registries searched	X
	• Search software used, name and version, including special features used (eg, explosion)	X
	• Use of hand searching (eg, reference lists of obtained articles)	X
	• List of citations located and those excluded, including justification	X
	• Method of addressing articles published in languages other than English	X
	• Method of handling abstracts and unpublished studies	X
	• Description of any contact with authors	X
Study Selection (Describe)	• Types of study designs considered	X
	• Relevance or appropriateness of studies gathered for assessing the hypothesis to be tested	X
	• Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	X
	• Documentation of how data were classified and coded (eg, multiple raters, blinding, andinterrater reliability)	X
	• Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	X
	• Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	X
	• Assessment of heterogeneity	N/A
	• Statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models,or cumulative meta-analysis) in sufficient detail to be replicated	N/A
Results (Present)	• A graph summarizing individual study estimates and the overall estimate	X
	• A table giving descriptive information for each included study	X
	• Results of sensitivity testing (eg, subgroup analysis)	X
	• Indication of statistical uncertainty of findings	X
Discussion (Discuss)	• Strengths and weaknesses	X
	• Potential biases in the review process (eg, publication bias)	X
	• Justification for exclusion (eg, exclusion of non–English-language citations)	X
	• Assessment of quality of included studies	X
	• Consideration of alternative explanations for observed results	X
	• Generalization of the conclusions (ie, appropriate for the data presented and within the domainof the literature review)	X
	• Guidelines for future research	X
	• Disclosure of funding source	X
N/A: Not Applicable		

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A systematic review of the influence of occupational organophosphate pesticides exposure on neurologic impairment

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Authors:

Noriko Takahashi

Masahiro Hashizume

Authors' Institutions:

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Address for correspondence:

Noriko Takahashi

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Tel: (+81) 95 819 7764 Fax: (+81) 95 819 7844

E-mail: pediatric.nagasaki@gmail.com

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ABSTRACT

Objective: The aim of this study was to conduct a systematic review of published literature and to estimate whether or not there is a causal relationship between occupational exposure to Organophosphate pesticides (OPs) and either neurologic impairment or depressive symptoms.

Data sources: EMBASE, MEDLINE, Global Health, and PsycINFO (1980 to April 2014).

Setting: Observational studies (cross-sectional, cohort, and case-control studies) with both exposed and unexposed groups.

Participants: People who occupationally use OPs more than one month and their family.

Primary outcome: Results of neurological core test batteries or depressive symptoms such as headaches, anxiety, and dizziness.

Study appraisal and synthesis methods: After an extensive search of various literature databases, one author screened titles and abstracts, searched the relevant publications manually, and conducted data extraction. All extracted data from the selected articles were synthesized for analysis. Quality appraisal was conducted using Newcastle Ottawa Scale.

Results: Of the 1024 articles retrieved by database search, 24 studies that met inclusion and exclusion criteria were selected for analysis. Of the selected studies, 17 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10 studies), UK (four studies), Africa (four studies), Asia (three studies), Europe (two studies), and South America (one study). Each of the included studies used different exposure

and outcome assessments such as neurologic scores and depressive symptoms, making it difficult to compare the results exactly. Most studies showed that exposed groups had poorer results than unexposed groups; however, because of inconsistent neurological test batteries there was not enough pooling evidence to conduct a meta-analysis.

Conclusion: The findings of this literature review indicate that it is a necessary to standardize the neurological or neuropsychological test battery and methods of measuring exposure to OPs.

Trial registration: Not applicable.

ARTICLE SUMMARY

Article Focus

- To systematically review epidemiological studies that examine adverse effects on the human central nervous system (CNS) by exposure to organophosphate pesticides (OPs).

Key messages

- OPs have been used widely all over the world for agricultural or industrial use.
- Many studies have examined acute health problems caused by OPs; however, few studies have investigated negative effects caused by occupational OPs exposure.

Strengths and limitations of this study

- The article represents a systematic review of epidemiological studies on adverse effects on the human CNS by occupational OPs exposure, with a quality appraisal of each study.
- The article identifies problematic issues of exposure and outcome assessments.
- Meta-analysis could not be applied because only a small number of pooled studies were available.
- In some studies it was difficult to judge negative effects caused only by OPs, because mixed pesticides were used.

INTRODUCTION

Ever since organophosphate pesticides (OPs) were developed, they have been used to combat insects for public health purposes and to support agricultural productivity and manufacturing processes. Because pesticides are also one of the leading suicide methods, a large number of epidemiological studies have investigated the relationship between high level OPs exposure such as pesticide poisoning and accidents and acute health effects. It has been reported that high level OPs exposure is significantly related to neurological or neuropsychological impairment (1, 2). In contrast, few studies have reported associations between occupational or cumulative OPs exposure and negative effects on human health, although some research has examined the negative influence on young children of cumulative OPs exposure (3, 4) and others have investigated relationships between reproductive health and occupational OPs exposure (5-7). High level OPs exposure are known to have adverse effects on the human CNS, therefore, occupational or cumulative OPs exposure also has the potential to negatively affect the CNS. However, very few epidemiological studies that have assessed the relationships between occupational OPs exposure and neurologic or mental problems have been published. The objective of this systematic review is to verify whether or not occupational OPs exposure negatively affects the human CNS. To investigate this further, we summarized the epidemiological evidence for the relationship between occupational OPs exposure and mental and neuropsychological aggression, especially for occupational OP users, and some of the limitations associated with the various studies are discussed.

MATERIALS AND METHODS

Searching strategy for identification of published studies

We searched the published literature using the OvidSP search software (8) to select relevant observational studies. A geographical restriction was not imposed; however, the search was restricted to studies published from 1980 to 2014. Population-based case-control studies were excluded from the systematic review because it was difficult to assess accurate exposure doses for these studies. Because various pesticides including OPs are currently easily available to everyone, it is highly likely that these pesticides have been obtained for personal use. For this reason, it is almost impossible to obtain past records of pesticide use by every individual. The literature search was limited to studies in humans and to reports published in English, and the review was limited to epidemiological studies. Moreover, unpublished studies and grey literature (literature that has not been formally published) were not searched in this systematic review; therefore we did not contact authors to find unpublished studies. Studies investigating OPs exposure through food and water contamination were also excluded. A search of the following four databases was carried out: EMBASE Classic + EMBASE (1980 to Week13 2014); Ovid MEDLINE(R) (1980 to Week13 2014); Global Health (1980 to Week12 2014); and PsycINFO (1980 to Week14 2014).

A combination of free-text terms and explore terms was used to identify relevant articles. For exposure, the following search keywords were used: organophosphate*, organophosphorous,

pesticide*, or insecticide*, and organophosphate pesticide (explore map term). For outcome, the following search keywords were used: neuro*, psychiatr*, psycholog*, mental health, mental illness, mental disorder, or depressi*, depression (explore map term), and mental health (explore map term). For subjects, the following search keywords were used: occupation*, agricultu*, or farm*. For study design, the following search keywords were used: epidemiolog*, cohort, cross-sectional, or case-control, and epidemiology (explore map term). An initial systematic search in the titles and abstracts was conducted using a combination of all these search terms. A second manual search of the reference lists from the selected relevant articles was performed to explore or retrieve articles found in the initial search in order to find as many available studies as possible.

Criteria for selecting studies for review

Only original research articles meeting the inclusion and exclusion criteria described below were used in the final review.

Inclusion criteria:

1. Study design

a) Must be observational studies: cross-sectional, cohort, and case-control studies.

b) Studies must have both exposed and unexposed groups.

2. Subjects

a) The subjects in the exposed group either must use OPs occupationally, or there must be a probability of being exposed to OPs during their work.

b) The families of occupational OP users can be treated as subjects.

3. Exposure

a) Subjects must be exposed to OPs for at least one month.

b) Seasonal workers who used OPs for more than one month must be included.

4. Outcome

Studies must have carried out some tests to assess damage to the CNS or have conducted a survey or an interview to identify depressive symptoms.

5. Exposure-outcome association

Results must be reported as some types of relative risks or mean scores.

Exclusion criteria:

1. Study design

Experimental and laboratory based studies including animal studies were excluded. Population-based case-control studies were excluded.

2. Subjects

Studies of mainly patients of pesticide poisoning were excluded.

3. Exposure

Studies that did not specify the type of pesticides were excluded.

4. Outcome

Studies examining damage of the peripheral nervous system due to OPs exposure were excluded.

5. Language

Studies published in a language other than English were excluded.

Definitions used for the review

Definition of cumulative exposure

a) People who used OPs in their jobs for at least one month and had the probability of inhaling ambient OPs and absorbing OPs by spraying and touching.

b) Families of OP users were included as subjects because they may have been exposed to OPs by washing clothes contaminated by OPs and/or by touching OP users.

Definition of poor mental health

A) Neurological or neuropsychological impairment

a) People who had poorer results in neurological or neuropsychological test batteries than healthy people of the same age.

b) People who had short-memory loss; for example, people who had experienced memory loss of six to three months duration.

B) Depressive Symptom

c) People who, regardless of their age, had chronic depressive symptoms including headache, fatigue, dizziness, sleepless, and eye problems.

d) People who were diagnosed with depression by clinical doctors.

Study selection process

Using the search terms listed above, a total of 1024 references were obtained: 515 from EMBASE Classic + EMBASE, 31 from PsycINFO, 196 from Global Health, and 282 from Ovid MEDLINE(R) (Figure1). However, 77 animal studies, 90 studies not in English studies, and 12 studies that did not meet the time restrictions were excluded. Of the remaining 845 studies, 516 were excluded because of duplications. A manual search of the titles and abstracts of the remaining 329 references excluded a further 272 studies. The 21 remaining articles were fully reviewed, after which 12 studies were deemed to meet inclusion and exclusion criteria (9-20). In addition, 12 articles identified by the manual search were added to the systematic review (Figure1). To include as many relevant studies as possible, studies published before 1980 that were found by the manual search were included to the list for review. Finally, these 24 studies were selected for data extraction (9-32).

Data extraction, synthesis, and analysis

Data extraction forms were created to compare relevant data collected from each of the 24 studies. The following data were extracted to assess heterogeneity of the included studies: title, authors, year

published, number of subjects in the exposed and unexposed groups, occupation, and demographic information such as mean age, sex, smoking status, and geographical area. In addition, the following data were extracted to assess confounding factors and statistical models among the included studies: inclusion and exclusion criteria such as first language, alcohol consumption, injury experience, confounding factors, and statistical methods used. The following data were extracted to assess exposure and outcomes: types of pesticides, exposure assessment, and outcome assessment to measure the neurologic or neuropsychological ability, and results obtained. Tables containing the data that were obtained using the data extraction forms were constructed and analyzed. P-values and 95% confidence intervals (95%CIs) were elicited from the articles to judge statistical uncertainty. When a study had investigated depressive symptoms, the information was collected and a table was constructed. Impact and statistical magnitude of depressive symptoms were represented using plus or minus signs '++', '+', and '−', based on the P-value or 95%CI of the studies. All data extraction, coding, and quality appraisal were conducted only by the first author; therefore, no disagreement events occurred.

Quality appraisal

The quality of the 24 studies was appraised using a scale adapted from the 'Newcastle/Ottawa Scale (NOS)'(33) (The appraisal standard of NOS is shown in Appendix A). Based on the NOS, each study was evaluated using the point system. When a study included relevant information that could be

associated to the NOS, one point was added. Five items in cross-sectional studies and eight items in cohort and case-control studies that could be related to the NOS were identified. Therefore, cross-sectional studies assigned 5, 4, 3, or 0−2 points were evaluated as very good, good, satisfactory, or unsatisfactory studies, respectively. Similarly, cohort/case-control studies with 7−8, 5−6, 4, and 0−3 points were identified as very good, good, satisfactory, or unsatisfactory, respectively.

RESULTS

As a result of the search strategy described in the Materials and Methods section, 12 studies were identified from the database search and another 12 studies were found after a manual search. These 24 articles, published between 1975 and 2010, met all the inclusion and exclusion criteria. A summary of the characteristics of the 24 selected articles is shown in Table 1.

Study design and geographical area

Of the selected studies, 17 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10 studies), UK (four studies), Africa (four studies; two in South Africa, one in Egypt, and one in Kenya), Asia (three studies; two in India and one in Sri Lanka), Europe (two studies; one in Spain and one in Poland), and South America (one study; Ecuador).

Characteristics of subjects

Because the subjects were limited to people who had the probability of being occupationally exposed by OPs, the majority of the participants (60−70%) were men. Most of the time, agricultural work such as pesticide application and farming is performed predominantly by men. Six of the 24 studies included both male and female subjects (9, 11, 17, 25, 27, 32), and only one study used all female subjects in both the exposed and control groups (21). In 13 of the studies the mean age of the exposed subjects was in the 30s, in six studies the mean age was in the 40s (9, 14, 15, 19, 20, 31), and in two studies the mean age was in the 50s (13, 17). The mean age in two studies was 29, very close to thirty (25, 32). One of the studies did not report detailed demographic data of the participants (10).

Source of recruitment and sample size

Ten out of the 24 studies were on pesticide applicators including private, commercial, and tree, fruit, and vegetable applicators. Five and three studies were on farmers and sheep farmers, respectively, and two studies were on factory workers and greenhouse workers. One study investigated depressive symptoms in the spouses of OPs users. In the study by Korsak et al. (22), the specific occupation of the population in the study was not stated, however, the subjects had experienced occupational OPs exposure. The number of subjects in the exposed groups varied from 16 to 2,051, while the control groups had a wider range of subjects (16 to 27,023).

Table1 Findings reported in epidemiological studies into occupational low level OPs exposure and mental illness

	Author	Study Design	Country	Exposed Population(No)	Chemical	Exposed Assessment	Comparison Group
1	Albers et al (9)	CO	USA	Chemical workers(53)	OP	Industrial HR,AChE INH	Similar workers, not exposed(60)
2	Bazylewicz-Walczak et al (21)	CO	Poland	Greenhouse workers(26)	OP	DR	Greenhouse workers, not exposed(25)
3	Beseler et al (10)*	NC/ CO	USA	Case**: Spouses of private applicators with depressive diagnoses(2,051)	OP	QU or IN	Control: Spouses of private applicators without depressive diagnoses (27,023)
4	Cole et al (11)	CR	Ecuador	Farmers, some applicators(144)	OP,CAR, FNG	IN, QU, AChE INH	Local Population(72)
5	Daniell et al (12)	CO	USA	Farm worker applicators(49)	OP	QU, AChE INH	Slaughterhouse workers(40)
6	Dassanayake et al (13)	CR	Sri Lanka	Vegetable farm workers (38)	OP	N.A.	hospital labours(35)
7	Farahat et al (14)	CR	Egypt	Farm workers(52)	OP	AChE INH	Local Population(50)
8	Fiedler et al (15)	CR	USA	Tree fruit farmers (57)	OP	QU, lifetime exposure metric	Cranbury/blueberry growers(low exposed), hardware storeowners(unexposed) (42)
9	Korsak et al (22)	CR	USA	Occupational exposure(16)	OP, CAR, OC	AChE INH	Local Population(low exposure)(16)
10	Levin et al (23)*	CR	USA	Pesticide applicators(24)	OP	IN, AChE INH	Farmers(24)
11	London et al (16)	CR	South Africa	Fruit farm pesticide applicators(163)	OP	QU(job-matrix)	Farm workers, not applicators(84)
12	London et al(24)*	CR	South Africa	Fruit farm pesticide applicators(164)	OP	QU (job-matrix)	Farm workers, not applicators(83)
13	Maizlish et al (25)	CR	USA	Pesticide applicators(46)	OP	UM, DR	Non-applicators(56)
14	Misra et al (26)*	PR	India	Pesticide applicators(22)	OP	AChE INH	Hospital labours(20)

15	Ohayo-Mitoko et al (27)*	CO	Kenya	Farm worker applicators(256)	OP, CAR	AChE INH	Farm workers(low exposure)(152)
16	Rodnitzky et al (28)	CR	USA	Pesticide applicators(23)	OP	AChE INH	Farmers(23)
17	Roldan-Tapia et al (18)	CR	Spain	Greenhouse workers(40)	OP, CAR	QU, AChE INH	Local Population(26)
18	Ross et al (17)	CO	UK	Sheep farmers(127)	OP	IN	Police workers(78)
19	Rothlein et al(32)	CR	USA	Farm workers(96)	OP	UM, House dust	Workers in hotels and tourist industry(45)
20	Srivastava et al(29)	CR	India	Manufacture workers(59)	OP	AChE INH	Manufacture workers, not exposed(17)
21	Steenland et al(30)	CR	USA	Termiticide applicators(191)	OP	IN,UM	Friends, blue collar workers(189)
22	Stephens et al (19)	CR	UK	Sheep farmers(146)	OP	QU	Quarry workers(143)
23	Stephens et al (31)	CR	UK	Sheep farmers(77)	OP	QU, UM	Quarry workers(69)
24	Stephens et al (20)	CR	UK	Orchard applicators(37)	OP	IN,QU	Construction workers,pig farmers(57)

Study Design CR: Cross-sectional, CO: Cohort, NC: Nested case-control, PR: Prospective study

Chemical OP: Organophosphates, OC: Organochlorines, CAR: Carbamates, FUN: Fungicides, AChE: Acetylcholinesterase

Exposed Assessment AChE INH: AChE inhibition, DR: Dermal and respiratory absorption, IN: Interview , QU: Questionnaire , HR: Hygiene records UM: Urinary metabolites

*Studies that included depressive symptoms for outcome assessments.

**Cases were defined as female spouses of private applicators who responded 'yes' to the question "Has a DOCTOR ever told you that you had been diagnosed with depression requiring medication?" Controls were female spouses who responded 'no' (10).

Exposure assessment

Exposure assessment in the included studies could be divided, for the most part, into five patterns: indirect assessment using, for example, an interview or questionnaire; direct assessment including a measurement of urinary metabolites and acetylcholinesterase (AChE) levels in the blood or a measurement of ambient OPs using a patch and a pump; combination of direct and indirect methods; combination of a biomarker and OPs exposure levels included in house dust; and combination of biomarkers and ambient OP levels. Seven of the 24 studies used indirect methods, and six studies used blood AChE inhibition levels to measure AChE levels in the blood as an exposure indicator. Six studies used a combination of indirect methods and biomarkers, three studies used biomarkers and the ambient OP levels, one study used a biomarker and house dust. The remaining study did not mention any exposure assessment methods. In all the studies that used urinary metabolites as exposure assessment, the results were presented as the sum of dialkylphosphates (DAP) (i.e. the sum of six DAP metabolites: DMP (dimethylphosphate), DMTP (dimethylthiophosphate), DMDTP (dimethyldithiophosphate), DEP (diethylphosphate), DETP (diethylthiophosphate), and DEDTP (diethyldithiophosphate)) (25, 30-32).

Outcome measurements

Two different outcome measurements were used in the studies; one measured neurological impairment and the other assessed depressive symptoms. Of the 24 studies, 19 used cognitive function tests to investigate negative neurologic influences caused by OPs exposure.

Associations between outcome and exposure

Ten of the 19 studies that investigated cognitive impairment mentioned that at least one measure outcome showed more impairment in the exposed groups; however, these observations were not significant ($P<0.05$). Seven of the studies reported some significant positive associations of exposure with poor outcome ($P<0.05$); however, even in these cases, the significant decrements were observed only in some of the neurologic tests, mainly in the Digit Span and Santa Ana Dexterity tests. Indeed, there are several versions of these neurologic tests and the significance of the scores often depended on the versions of the tests that were used. Five studies used the Wechsler Adult Intelligence Scale (WAIS or WAIS-R) (34, 35), four studies used the Neurobehavioral Evaluation System (NES) (36), two studies used the World Health Organization Neurobehavioral Core Test Battery (NCTB) (37, 38), and the remaining

eight studies used their own scales.

Five studies adopted depressive symptoms as outcome measurements, as shown in Table2; however, the symptoms used in the studies were not standardized.

Table2 Summary of depressive symptoms used as outcomemeasurements

Reference	Results obtained	Impact of outcomes
Beseler et al 2006(10)	Depression due to doctor's diagnosis was not significantly related to low (OR 1.09; 95%CI 0.91, 1.31) or high (OR 1.09; 95%CI 0.91, 1.31) cumulative exposure.	-
Levin et al 1976(23)	Anxiety score of the pesticide applicators was significantly higher (P<0.05) than that of the farmers. However, there was no significant difference in measures of depression.	++
London et al 1998(24)	Dizziness, sleepiness, and headache had a significantly higher overall neurological symptom score (P<0.05).	++
Misra et al 1985(26)	Common symptoms were Headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%) and no neurologic change was seen.	-
Ohayo-Mitoko et al 2000(27)	A significant change in symptom prevalence was found for the respiratory (2.48% CI (0.78, 5.38) and central nervous system (2.56% CI (0.99, 6.62), but in terms of skin symptoms, and eye symptoms, there was no statistically significant change.	++

OR=Odds Ratio ++: Statistically significant (P<0.05), -: Not statistically significant

Statistical analysis

Sixteen studies used logistic regression, and the remaining eight used other statistical tests including X^2-test and t-test. Only one study adjusted for sex in the logistic

regression. Fourteen of the 24 studies adjusted for age, and 12 adjusted for education in the statistical analysis. However, only five studies adjusted for alcohol consumption before carrying out the statistical analysis, and only two studies adjusted for first language.

Methodological quality appraisal

Based on NOS, five of the 24 studies were of very good quality, 10 were of good quality, and the remaining nine were either satisfactory or unsatisfactory. Most studies with unsatisfactory scores either were carried out before 1990 or were performed in some of the less developed countries. In particular, the methods of recruitment of subjects, controlling for confounders, and outcome assessment were not appropriate. For example, in some studies, all of the participants were volunteers (14, 28) and in another study, the subjects were not representative of the community from which they were recruited (factory workers) (29). In addition, in the unsatisfactory studies, how the outcome was assessed was not described, and methods needed to avoid confounders such as stratification and regression were not used. None of the cohort studies were assessed as very good quality because most of them did not have a long enough follow-up duration (in five studies, the duration was less than six months) and the

selected subjects were not fully representative of the target community. Moreover, the methods of outcome assessment were not described in most of the cohort studies.

Data synthesis

The results of the neurologic tests used in the studies are summarized in Table3. As can be seen, the test batteries differed from study to study. The commonly used test batteries in NCTB, NES, and WAIS were Symbol-Digit and Digit Span Forward and Backward. However, some studies that adopted NES and WAIS to measure neurologic impairment implemented only a few subsets in the trials. Among the 13 studies that used a Symbol-Digit test, four used NES and unknown tests, two used WAIS and WAIS-R, and one used a Polish NCTB. Among the studies that used Digit Span Forward and Backward tests, some studies performed both tests, while the others did only one of the tests as shown in Table3. Overall, only four of the studies used the same test battery in NES and WAIS. Although three studies apparently used the same scoring systems, one of the scores was completely different from the scores in the other two studies. For example, the scores in the study by Stephens et al. (31) were 24.22 and 21.01 in the exposed and the control groups respectively, whereas the scores reported by Daniell et al. and Stephens et al. were much lower and between 2.23 and 3.55 (12, 20). Similarly,

the mean scores reported by Bazylewicz-Walczak et al. (21) were higher, 45.50 and 49.40, while the mean scores reported in the other studies were smaller, 2.28 and 2.23 in the WAIS (25). In consideration of insufficient number of studies and possible systematic differences in the population characteristics and/or in the measurement procedures between the studies, we decided not to conduct a meta-analysis.

Table3 Summary of the neurologic test batteries used in some of the studies

Reference	Types of neurologic tests	Symbol Digit	Digit Span	Santa Ana	Simple Reaction Time	Syntactic Reasoning(s)
Bazylewicz-Walczak et al 1999(21)	Polish NCTB/WAIS (Symbol Degit)	nd	nd	nd	**	nd
Cole et al 1997(11)	NCTB	nm	nm	nm	nd	nd
Daniell et al 1992(12)	NES	*	nd	nd	nd	nd
Farahat et al 2003(14)	Unknown	***	***(f)[1] ***(b)[2]	nd	nd	nd
Fiedler et al 1997(15)	WAIS-R	*	*	nd	***	nd
London et al 1997(16)	WAIS-R	nm	nm	***	nm	nd
Maizlish et al 1987(25)	WAIS	***	nd	nd	nd	nd
Roldan-Tapia et al 2005(18)	WAIS	*** [3]	*** [3]	nd	nd	nd
Ross et al 2010(17)	WAIS	nd	***	nd	nd	nd
Rothlein et al 2006(32)	Unknown	*	*(f)[1] ***(b)[2]	nd	*	nd
Srivastava et al 2000(29)	Unknown	***	***	nd	nd	nd
Steenland et al 2000(30)	NES	*	*	nd	*	nd
Stephens et al 1995(19)	Unknown	***	*	nd	***	**
Stephens et al 1996(31)	NES/ACT	nm	nm	nd	nm	nm

Stephens et al 2004(1)	NES/ACT	*	*	nd	*	*** (ACTS)

***P<0.05, **0.05≦P<0.1, *P>0.1

The exposed groups were slower or had poorer outcomes than the control groups

[1](f) Digit Span Forward

[2](b) Digit Span Backward

[3]Whether the obtained results were positive or negative was not reported in the study.

nd: Subsets of neurological tests were not performed.

nm: Subsets of neurological tests were performed but P-values were not reported.

DISCUSSION

The systematic keyword and manual searches of the published literature identified 24 epidemiological studies that examined the relationship between OPs and CNS. When the relevant information was assessed, two main findings were obtained, one was the method of exposure assessment, and the other was the method used for the outcome measurement. For exposure assessment, the measurement methods were categorized as direct, indirect, and a combination of direct and indirect. For the outcome measurements, two main assessments were used, neurologic impairment and depressive symptoms.

Exposure assessment

Exposure assessment was not used for group allocation in all the studies; rather, it was implemented to measure how much subjects were exposed and the outcomes of the neurobehavioral tests. Different exposure assessment methods were used in each study,

which made it difficult to accurately compare the studies. In addition, there seemed to be methodological imperfections in both the direct and indirect methods. For example, in one study, an interview and questionnaire were used for recruited subjects over 60 years old who had been retired for 11 years (17). This method is subject to recall bias because the rate of cognitive impairment is likely to have increased as the subjects aged. However, other indirect methods, especially extensive history records of pesticide use could be considered as a proxy of how much OPs might have accumulated in the body, thus records of this type can be used to estimate the amount of OPs by long-term exposure, even though there may be some recall bias. For the direct methods, DPA or urinary metabolites was used as an exposure index in the study; however, DPA is metabolized rapidly and excreted (6). On the contrary, blood AChE levels take approximately one week to become normal (39); hence, although blood AChE levels cannot be used to assess the accumulation of OPs in body tissues over a long time, it can be used to assess short-term exposure. To minimize measurement errors, a mixed method for the assessment of short-term and long-term exposure should be established.

Outcome assessment

The main problem in analyzing the outcome measurements was the inconsistencies in

neurologic test batteries. Various versions of the neurologic tests were used in the studies and the content of the tests differ slightly in each study (Table3). Therefore, only a few tests were common across some of the studies, which made it difficult to compare the studies. Further, a meta-analysis could not be applied because of the insufficient number of studies. Meta-analysis could have been performed by dividing the results into subgroups; however, the results could be highly misleading because of loss of power (40). In terms of depressive symptoms, the outcome assessment was again different in each study. For instance, one study used the proportion of headaches, while another used dizziness and sleepiness as the main outcomes. To gain better insights into whether occupational OP exposure can negatively affect the human CNS, at the very least, neurologic test batteries should be standardized and guidelines for measuring of neurologic symptoms should be set for all future epidemiological studies. Furthermore, although some studies mentioned the possible relationship between OPs exposure and confounding factors such as age and education, statistical tests between the exposed and control groups were not performed in these studies. These inconsistencies make it difficult to compare the neurologic impairment outcomes among the studies.

Study design

Although 17 of 24 studies were cross-sectional studies, longitudinal or cohort studies are more appropriate, because agricultural work using pesticides is easily influenced by seasonality. One research regarding reproductive health by OPs exposure stated that sperm concentration and counts are negatively affected in spring, peak season, rather than winter (5). Therefore, the effect on the CNS could also be affected by seasonality.

Sources of possible biases

Only published studies written in English were searched, thus publication bias could have occurred. In future studies, non-English studies and unpublished studies should be included to reduce publication bias. In trials that included foreign workers, first language and education levels could be considered as possible biases because there is a possibility that non-native subjects did not fully understand the content and instructions for the tests, which could lead to them obtaining a lower score than native speakers. Additionally, the education systems in developed and less developed countries could be very different. Nowadays, developed countries such as USA and the Gulf countries have accepted foreign workers as an important part of the workforce (12, 32, 41). These factors needed to be adjusted carefully in the sampling and analytical stages of the study; however, only two of the selected studies mentioned first language in their

statistical analyses (12, 31). Occupation could also contribute to selection bias because, for example, a police officer or a construction worker would have a higher probability of experiencing loss of consciousness due to accidents than workers with different occupations (17).

Possible confounders

Apart from common confounders such as age and education, head injury and alcohol consumption could be other confounders, because they can cause neurologic impairment due to memory deterioration. Although some of the studies adjusted for alcohol consumption in the analysis (10, 12, 16, 24, 25), no study adjusted for head injury. Furthermore, nutrition status including vitamin deficiency can also be relevant to the outcome of neuropsychological tests (16, 24). Thus, factors other than the common confounders that could negatively affect cognitive function should be adjusted for in the analysis.

Strengths and limitations of this review

A major strength of this systematic review is that the characteristics of the selected studies were summarized using tables, and limitations of the exposure and outcome

assessments used in these studies were identified mainly on the basis of the constructed tables. Furthermore, the systematic review allowed us to propose recommendations that will be useful for standardizing future epidemiological research.

All of the selected studies were relevant to occupational OPs exposure; however, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides that are commonly used in agriculture are usually mixtures of different pesticides, which are used to increase their effect. Four of the 24 selected studies used a combination of OPs, organochlorines, carbamates, and fungicide; hence, the effect of only occupational OPs exposure could not be measured in these studies. In the outcome assessments, different neurological types of tests were used, consequently, the lack of pooling evidence meant that a meta-analysis could not be performed. Furthermore, the exclusion of studies written in languages other than English is another limitation of this review, and literature retrieval by only the first author could have introduced some bias into the selection of the studies.

CONCLUSION

The items tested in the neurological or neuropsychological test batteries, and the estimates of OPs exposure were inconsistent because they depended on the preferences

of the investigators. For future studies, it would be best to standardize the neurological and neuropsychological test types, test batteries, and the methods used to measure OPs, to enable precise comparisons of results and pooling of evidence from a large number of studies for future analyses. However, this may be difficult to achieve in practice because OPs are used in differing settings around the world, and education systems vary considerably between countries.

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FOOTNOTES

Contributors NT conceived the study design and participated in protocol development, literature searching, data extraction, data analysis, and drafted the manuscript. MH critically reviewed the draft and contributed to the manuscript revisions.

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Data sharing statement No additional data are available.

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FIGURE LEGEND

Figure 1: Flow diagram of search and review process

Title: A systematic review of the influence of occupational organophosphate pesticides exposure on neurologic impairment

Authors:

Noriko Takahashi[†]

Masahiro Hashizume[†]

Authors' Institutions:

[†] Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, ~~Nagasaki City,~~ Nagasaki~~,~~ 852-8523, Japan

Address for correspondence:

Noriko Takahashi~~Furuoya~~

Department of Pediatric Infectious Diseases, Institute of Tropical Medicine, Nagasaki University

1-12-4 Sakamoto, ~~Nagasaki City,~~ Nagasaki 852-8523, Japan

Tel: (+81) 95 819 7764 Fax: (+81) 95 819 7844

E-mail: pediatric.nagasaki~~furuoyanoriko~~@gmail.com

Key words: Organophosphate, Pesticides, Central Nervous System, Neurologic, Impairment

Word count, main text: 41732~~3~~

Number of Tables/Illustrations: 4~~3~~4

Number of References: 4134~~7~~8

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ABSTRACT

~~**Background:** Organophosphate pesticides (OPs) are widely used widely;: however, only a few epidemiological studies have investigated the association between neurologicalbehavioral or neuropsychological effects and occupational OP exposure.~~

Objective: The aim of this study was to conduct a systematic review of ~~the~~ published literature~~s~~ and to estimate whether or not there is a causal relationship between occupational exposure to Organophosphate pesticides (OPs) and either neurologic impairment or depressive symptoms.

Data sources: EMBASE, MEDLINE, Global Health, and PsycINFO (1980 to April 2014).

Setting: Observational studies (cross-sectional, cohort, and case-control studies) with both exposed and unexposed groups.

Participants: People who occupationally use OPs more than one month and their family.

Primary outcome: Results of neurological core test batteries or depressive symptoms such as headaches, anxiety, and dizziness.

Study appraisal and synthesis methods~~**Method**~~: After ~~A~~an extensive search of various literature databases, one author screened titles and abstracts, searched the relevant publications manually, and conducted data extraction. ~~was conducted, and the relevant publications were then manually searched manually.~~ All ~~the relevant data wereas~~ extracted data from the selected articles were~~and~~ synthesized for analysis. Quality appraisal was conducted using Newcastle Ottawa Scale. ~~Meta-analysis was implemented using mean scores of the neurologic tests and depressive symptoms.~~

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Results: Of the 1024 articles retrieved by database search, 24~~Twenty three~~ studies that met inclusion and exclusion criteria were selected for analysis. Of the selected studies, 17~~6~~ were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10~~nine~~ studies), UK (four studies), Africa (four studies), Asia (three studies), Europe (two studies), and ~~one in~~ South America (one study). E~~The E~~ach of the included studies~~y~~ used different exposure and outcome assessments such as neurologic scores and depressive symptoms, ~~thus~~ making it difficult to compare the results exactly. ~~The m~~Most studies showed that ~~the~~ exposed groups had poorer results than ~~the~~ unexposed groups; ~~;~~ however, because of inconsistent neurological test batteries there was not enough pooling evidence to conduct a meta-analysis~~evidence based on the results of the meta-analysis was weak~~.

Conclusion: The findings of this literature review indicate that ~~there~~it is a necessary~~necessity~~ to standardize the neurological~~behavioral~~ or neuropsychological test battery and methods of measuring ~~OPs~~ exposure to OPs.

Trial registration: Not applicable.

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~~there might be a causal relationship between occupational exposure to OPs and neurological impairment or depressive symptoms.~~

ARTICLE SUMMARY

Article Focus

- To systematically review epidemiological studies that~~which~~ examine adverse effects on the human central nervous system (CNS) by exposure to organophosphate pesticides (OPs).

Key messages

- OPs have been ~~widely~~ used widely all over the world for agricultural or industrial use.
- Many ~~There are a plenty of~~ studies have~~which have~~ examined acute health problems caused by OPs~~:~~, however, few studies have investigated negative effects caused by occupational OPs exposure.

Strengths and limitations of this study

- The article represents a systematic review of epidemiological studies on adverse effects on the human ~~central nervous system~~CNS by occupational OPs exposure, with a quality appraisal of each study.
- The article identifies problematic issues of exposure and outcome assessments.
- M~~The m~~eta-analysis ~~was limited because each study used various outcome assessments~~could not be applied ~~due to~~because only a small number of ~~the~~ pooled studies were available.~

- In some studies~~,~~ ~~There~~ it ~~is~~was ~~a~~ difficult~~y~~ to judge negative effects caused ~~by~~ only by OPs, because mixed pesticides were used ~~in some studies~~.

INTRODUCTION

Ever since organophosphate pesticides (OPs) were developed, they have been used tofor combating insects for public health purposes and to support agricultural productivity and manufacturing processes. SinceBecause Ppesticides are also well-known as one of the leading suicide methods, and approximately three million cases of pesticide poisoning occur every year around the world. This is especially prevalent in Asian nations including Sri Lanka, China, and Malaysia (1). For this reason, a large number of epidemiological studies have investigated the relationship between high level OPs exposure such as pesticide poisoning and accidents and acute health effects(1, 2). and It has been reported that high level OPs exposure is significantly related to neurological or neuropsychological impairment (1, 2)((1, 2)2, 3). In contrast, few studies that have reportinvestigated associations between occupational or cumulative OPs exposure and negative effects on human health are available, even although. Although some research has examined the negative influence onto young children ofby cumulative OPs exposure (3, 4)((3, 4)4, 5) andor others have investigated relationships between reproductive health and occupational OPs exposure (5-7)((5-7)6-8).. Since hHigh levels OPs exposure providesare known to have adverse effects on the human CNScentral nervous system, therefore, occupational or cumulative OPs exposure has also has the potential to negatively affect the CNSit. However, there are very few epidemiological studies thatwhich have assessed the relationships between occupational OPs exposure and neurologic or mental problems have been published using epidemiological research. The objective of this systematic review is to verify

whether or not occupational OPs exposure ~~could~~ negatively affects ~~influence on~~ the human ~~central nervous system~~CNS. ~~In this systematic review~~To investigate this further ~~test the hypothesis~~, we summarized the epidemiological evidence for the relationship between occupational OPs exposure and mental and neuropsychological aggression, especially for occupational OP users, ~~is summarized,~~ and ~~and~~ some of the limitations associated with the various studies are discussed.

MATERIALS AND METHODS

Searching strategy for identification of published studies

~~A~~We search~~ed the published literature for observational studies was carried out~~ using the Ovid SP~~(8),~~ ~~a~~ search software (8) to select relevant observational studies~~, by the author~~. A ~~G~~geographical ~~and time~~ restriction~~s~~ ~~were~~was not imposed~~;~~, however, the search~~a published period~~ was restricted to studies published from 1980 to 2014~~Current~~. Population-based case-control studies were excluded from the systematic review because it was~~is~~ difficult to assess accurate exposure doses for these studies. Because ~~Currently,~~ various pesticides including OPs~~, currently,~~ are currently easily ~~-~~available to~~for~~ everyone, ~~and~~ ~~some people have a~~it is ~~possibility~~highly likely that ~~of using~~these pesticides have been obtained for personal use. ~~However~~For this reason, it is almost impossible to ~~comprehend exactly~~obtain past records of pesticide~~s~~ use by every ~~person~~individual. The literature search was limited to studies in humans and to reports published in English, and the review was limited to

epidemiological studies. Moreover, unpublished studies and grey literature (literature that has not been formally published)s were not searched in this systematic review;, therefore we did not make a contact with any authors to find out unpublished studies. Studies investigating OPs exposure through food and water contamination were also excluded. A search of the following four databases was carried out:

1. EMBASE Classic +plus EMBASE (198047 to 201Week13 20140 July 09);,
2. Ovid MEDLINE(R) (19850 to June Week 5 2010March Week134 2014);,
3. Global Health (198010 to June 2010Week12 2014);, and
4. PsycINFO (1980806 to July Week 1 2010April Week14 2014).

A combination of free-text terms and explore terms was used to identify relevant articles. For exposure, the following search keywords were used: organophosphate*, organophosphorous, pesticide*;, or or insecticide*, and organophosphate pesticide (explore map term);. pesticide (explore map term). For outcome, the following search keywords were used: neuro*, psychiatr*, psycholog*, mental health, mental illness, mental disorder, or depressi*, depression (Epidemiology) (explore map term), and mental health (explore map term). For subjects, the following search keywords were used: occupation*, agricultu*, or farm*. For study design, the following search keywords were used: epidemiolog*, cohort, or cross-sectional, or case-control, andor Eepidemiology (explore map term) were used as keywords. An initial systematic search in the titles and abstracts was conducted using a combination of all these search terms. A second manual search of the

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reference lists from the selected relevant articles was performed to explore or retrieve articles found in the initial search in order to find ~~out~~as many available studies ~~to the extent~~as possible.

Criteria for selecting studies for ~~the~~ review

Only original research articles meeting the inclusion and exclusion criteria described below were used in the final review~~result~~.

Inclusion criteria:

1. ~~–~~Study design~~:~~

a) Must be observational studies: cross-sectional, cohort, and case-control studies.

b) Studies must have both exposed and unexposed groups.

2. ~~–~~Subjects~~:~~

a) The subjects in the exposed group either must use OPs occupationally, or there must be a probability of being exposed to OPs during their work.

b) The families of occupational OP users can be treated as subjects.

3. ~~–~~Exposure~~–~~

a) Subjects must be exposed to OPs for at least one month.

b) Seasonal workers who used OPs for more than one month must be included.

4. ~~–~~Outcome~~–~~

Studies must have carried out some tests to assess damage toof the CNS ~~(Central Nervous System)~~ or have conducted a survey or an interview to identify depressive symptoms.

5. –Exposure-outcome association

Results must be reported as some types of relative risks or mean scores.

Exclusion criteria:

1. Study design

Experimental and laboratory based studies including animal studies were excluded. Population-based case-control studies were excluded.

2. Subjects

Studies of mainly patients of pesticide poisoning were ~~not~~ excluded.

3. Exposure

Studies thatwhich did not specify the type of pesticides were excluded.

4. Outcome

Studies examining damage of the peripheral nervous system due to OPs exposure were excluded.

5. Language

Studies published in a language other than English were excluded.

Definitions used for the review

Definition of cumulative exposure

a) People who used OPs in their jobs for at least one month and had~~ve~~ the~~a~~ probability of inhaling ambient OPs and absorbing OPs by spraying and touching.

b) Families of OP users were included as subjects~~,~~ because they may have been exposed to OPs by washing clothes contaminated by OPs and/or by touching OP users.

Definition of poor mental health

A) Neurological or neuropsychological impairment

a) People who had poorer results in neurological or neuropsychological test batteries than healthy people of the same age.

b) People who had short-memory loss~~;~~ for example, people who had experienced memory loss of six to three months duration.

B) Depressive Symptom

c) People who, regardless of their age, had chronic depressive symptoms including headache, fatigue, dizziness, sleepless, and eye problems.

d) People who were diagnosed with depression by clinical doctors.

Study selection process

Using the search terms listed above, a total of 1024~~592~~ references were obtained: 515~~276~~ from EMBASE~~mbase~~ Classic + EMBASE~~mbase~~, 31~~16~~ from PsycINFO, 196~~133~~ from Global Health, and

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282~~167~~ from Ovid MEDLINE(R)~~edline~~ (Figure1). However, 77 animal studies, ~~were excluded because they were not appropriate to test the hypothesis of this review. Furtheremore,~~ 90 studies ~~were~~ not in English studies, and 12 studies that did not meet ~~were removed due to~~ the time restrictions were excluded. Of the remaining 845 studies, 516~~197~~ ~~of 845592 references~~ were excluded ~~due to~~because of duplications~~es~~. ~~Of these 395 unique references that remained, 63 were not in English, and 32 were animal studies.~~ A manual search of the titles and abstracts of the remaining 32900 references excluded a further 272~~268~~ studies. The 21~~32~~ remaining articles were fully reviewed, after which 13~~2~~ studies were deemed to meet inclusion and exclusion criteria (9-20) ~~(8-20)((8-20)9-21)~~. In addition, 12~~0~~ articles identified by the manual search were added to the systematic review (Figure1~~See Appendix A for flow of study inclusion and exclusion diagram~~). To include as many relevant studies as possible, studies published before 1980 that were found by the manual search were included to the list for review. Finally, these 24~~3~~ studies were ~~identified~~selected ~~and used~~ for data extraction (9-32)~~(21)(8-31). ((8, 11, 13, 15, 19, 21-38)22-31).~~

Data extraction, synthesis, and analysis

Data extraction forms were created to compare relevant data collected from each of the 24~~3~~ studies. The following data were~~as~~ extracted to assess heterogeneity of the included studies~~as basic data~~:~~Extracted data included~~ title, authors, year published, ~~and the~~ number of subjects in the exposed and unexposed groups, occupation, and demographic information such as mean age, sex,

smoking status, and geographical area~~,~~. In addition ~~to basic data~~, the following data were~~as~~ extracted to assess confounding factors and statistical models among the included studies: inclusion and exclusion criteria such as first language, alcohol consumption, ~~and~~ injury experience, confounding factors, and statistical methods used.~~,~~ The following data were~~as~~ extracted to assess exposure and outcomes ~~assessment~~: types of pesticides, exposure assessment, and ~~statistical methods,~~ outcome assessment to measure the neurologic or neuropsychological ability, and results obtained. Tables containing the data that were~~as~~ obtained using the data extraction forms were constructed~~–~~ and analyzed~~.~~ –P-values and 95% ~~percent~~ confidence intervals (95%CIs) were elicited from the articles to judge statistical uncertainty. When a study had investigated depressive symptoms, the information was collected and a table was constructed. Impact and statistical magnitude of depressive symptoms were represented using plus or minus signs ~~including~~ '++', '+', and '–', based on the P-value or 95%CI of the studies. ~~Meta-analysis was carried out using mean scores of neuropsychological tests with STATA version 11.0.~~ All data extraction, coding, and quality appraisal were~~as~~ conducted only~~by only~~ by the first author~~;~~ therefore, no~~events in~~ disagreement events ~~were not~~ occurred.

Quality appraisal

The quality of the 24~~3~~ studies was appraised using a scale ~~that was~~ adapted from the 'Newcastle/Ottawa Scale (NOS)'(33) ~~(32)~~ (The appraisal standard of NOS is~~was~~ shown in Appendix A~~B~~). Based on the NOS, each study was evaluated using the point system. When a study included

relevant information that could be associated to the NOS, one point was added. FThere are five items in cross-sectional studies and eight items in cohort and case–control studies that couldcan be related to the NOS were identified. Therefore, cross-sectional studies assigned 5, 4, 3, or 0–2 points were evaluated as very good, good, satisfactory, or unsatisfactory studies, respectively. Similarly, cohort/case-control studies with 7–8, 5–6, 4, and 0–3 points were identified as very good, good, satisfactory, or unsatisfactory, respectively.

RESULTTLS

As a result of the search strategy described in the Materials and Methods section, 123 studies were identified from the database search and another 120 studies were found after a manual search. A total ofThese 243 articles, published between 1975 and 20100, met all the inclusion and exclusion criteria. A summary of the characteristics of the 243 selected articles is shown in Table 1.

Study design and geographical area–

Of the selected studies, 176 were cross-sectional and the remaining seven were cohort and nested case-control studies. The geographical areas included in the studies were USA (10nine studies), UK (four studies), Africa (four studies; two in South Africa, one in Egypt, and one in Kenya), Asia (three studies; two in India, and one in Sri Lanka), Europe (two studies; one in Spain and one in Poland), and one in South America (one study; Ecuador, one study).

Characteristics of subjects

Because the subjects were limited to people who had the probability of being occupationally exposed by OPs, the majority of the participants (60−70%) were men. Most of the time, agricultural work such as pesticide application and farming is ~~has been~~~~is predominantly~~ performed predominantly by men. Six~~Five~~ ~~out~~ of the 24~~3~~ studies included both male and female subjects~~,~~ ~~however, approximately 60 to 70 percent of the subjects were male~~ ~~(~~(9, 11, 17, 25, 27, 32)~~9, 21, 27, 29, 33)~~, and~~,~~ o~~O~~nly one study used all female subjects in both the exposed and control groups (21)~~(23)~~. In 13~~2~~ of the studies ~~T~~the mean age of the exposed subjects was in the ~~thirtie~~30s ~~in 12 studies~~, in six studies the mean age was in the 40~~forties~~ ~~(~~(9, 14, 15, 19, 20, 31)~~,9, 12, 16, 17, 24, 34)~~ and in two studies the mean age was in the ~~fiftie~~50s ~~(~~(13, 17)~~13, 21)~~. The mean age in two~~one~~ studies~~y~~ was ~~in the twenties, however, the mean age was~~ 29, very close to thirty ~~(~~(25, 32)~~27)~~. One of the studies~~y~~ did not report detailed demographic data of the participants ~~(10)~~(10).

Source of recruitment and sample size

Ten out of the 24~~3~~ studies were on pesticide applicators including private, commercial, and tree, fruit, and vegetable applicators. Five~~our~~ and three studies were on farmers and sheep farmers, respectively, and~~,~~ two studies were on factory workers and greenhouse workers. One study investigated depressive symptoms in the spouses of OPs users. In the study by Korsak et al. (22), the

specific occupation of the population in the study was not stated, however, the subjects had experienced occupational OPs exposure~~ (25(21))~~. The number of subjects in the exposed groups varied from 16 to 2,051, while the control groups had a wider range of subjects~~, with the figure ranging from~~ (16 to 27,023).

Table1 ~~Reported~~ Ffindings reported in ~~recent~~ epidemiological studies ~~regarding~~into occupational low level OPs exposure and mental illness

	Author	Study Design	Country	Exposed Population(No)	Chemical	Exposed Assessment	Comparison Group
1	Albers et al (9)~~(9)~~	CO	USA	Chemical workers(53)	OP	Industrial HR,AChE INH	Similar workers, not exposed(60)
2	Bazylewicz-Walczak et al (21)~~(23)~~	CO	Poland	Greenhouse workers(26)	OP	DR	Greenhouse workers, not exposed(25)
3	Beseler et al (10)~~(10)~~*	NC/ CO	USA	Case**: Spouses of private applicators with depressive diagnoses(2,051)	OP	QU or IN	Control: Spouses of private applicators without depressive diagnoses (27,023)
4	Cole et al (11)~~(33)~~	CR	Ecuador	Farmers, some applicators(144)	OP,CAR, FNG	IN, QU, AChE INH	Local Population(72)
5	Daniell et al (12)~~(20)~~	CO	USA	Farm worker applicators(49)	OP	QU, AChE INH	Slaughterhouse workers(40)
6	Dassanayake et al (13)~~(13)~~	CR	Sri Lanka	Vegetable farm workers (38)	OP	N.A.	hospital labours(35)
7	Farahat et al (14)~~(24)~~	CR	Egypt	Farm workers(52)	OP	AChE INH	Local Population(50)
8	Fiedler et al (15)~~(34)~~	CR	USA	Tree fruit farmers (57)	OP	QU, lifetime exposure metric	Cranbury/blueberry growers(low exposed), hardware storeowners(unexposed) (42)
9	Korsak et al (22)~~(25)~~	CR	USA	Occupational exposure(16)	OP, CAR, OC	AChE INH	Local Population(low exposure)(16)
10	Levin et al (23)~~(26)~~*	CR	USA	Pesticide applicators(24)	OP	IN, AChE INH	Farmers(24)
11	London et al (16)~~(18)~~	CR	South Africa	Fruit farm pesticide applicators(163)	OP	QU(job-matrix)	Farm workers, not applicators(84)
12	London et	CR	South Africa	Fruit farm pesticide applicators(164)	OP	QU (job-matrix)	Farm workers, not applicators(83)

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	al(24)~~(15)~~*						
13	Maizlish et al (25)~~(27)~~	CR	USA	Pesticide applicators(46)	OP	UM, DR	Non-applicators(56)
14	Misra et al (26)~~5(28)~~*	PR	India	Pesticide applicators(22)	OP	AChE INH	Hospital labours(20)
15	Ohayo-Mitoko et al (27)~~(29)~~*	CO	Kenya	Farm worker applicators(256)	OP, CAR	AChE INH	Farm workers(low exposure)(152)
16	Rodnitzky et al (28)~~(30)~~	CR	USA	Pesticide applicators(23)	OP	AChE INH	Farmers(23)
17	Roldan-Tapia et al (18)~~(14)~~	CR	Spain	Greenhouse workers(40)	OP, CAR	QU, AChE INH	Local Population(26)
18	Ross et al (17)~~(21)~~	CO	UK	Sheep farmers(127)	OP	IN	Police workers(78)
19	Rothlein et al(32)	CR	USA	Farm workers(96)	OP	UM, House dust	Workers in hotels and tourist industry(45)
20 ~~19~~	Srivastava et al(29)~~(31)~~	CR	India	Manufacture workers(59)	OP	AChE INH	Manufacture workers, not exposed(17)
21 ~~20~~	Steenland et al(30)~~(11)~~	CR	USA	Termiticide applicators(191)	OP	IN,UM	Friends, blue collar workers(189)
22 ~~1~~	Stephens et al (19)~~(12)~~	CR	UK	Sheep farmers(146)	OP	QU	Quarry workers(143)
23 ~~2~~	Stephens et al (31)~~(17)~~	CR	UK	Sheep farmers(77)	OP	QU, UM	Quarry workers(69)
24 ~~3~~	Stephens et al (20)~~(16)~~	CR	UK	Orchard applicators(37)	OP	IN,QU	Construction workers,pig farmers(57)

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Study Design CR: Cross-sectional, CO: Cohort, NC: Nested case-control, PR: Prospective study

Chemical OP: Organophosphates, OC: Organochlorines, CAR: Carbamates, FUN: Fungicides, AChE: Acetylcholinesterase

Exposed Assessment : AChE INH: AChE inhibition, DR: Dermal and Respiratory Absorption, IN: Interview , QU: Questionnaire , HR: Hygiene Records UM: Urinary metabolites.

*ArticlesStudies that including ed depressive symptoms for outcome assessments.

**Cases were defined as female spouses of private applicators who responded 'yes' to the question "Has a DOCTOR ever told you that you had been diagnosed with depression requiring medication?" Controls were female spouses who responded 'no' (10).

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Exposure assessment

Exposure assessment in the included studies could bewas divided, for the most part, into five patterns: indirect assessment using, for example, an interview or questionnaire; direct assessment including athe measurement of urinary metabolites and acetylcholinesterase (AChE) levels in the blood or a measurement of ambient OPs using a patch and a pump; a combination of direct and indirect methods; a combination of a biomarker and OPs exposure levels included in house dust;; and a combination of biomarkers and ambient OP levels. Seven out of the 243 studies used indirect methods, and six studies used blood AChE inhibition levels to measure AChE levels in the blood as an exposure indicator. Sixix studies used a combination of indirect methods and biomarkers, and three studies used biomarkers and the ambient OP levels, one study used a biomarker and house dust. The remaining study did not mention any exposure assessment methods. In all the studies thatwhich used urinary metabolites as exposure assessment, the results were presented as the sum of dialkylphosphates (DAP) (i.e. the sum of six DAP metabolites: DMP (dimethylphosphate), DMTP (dimethylthiophosphate), DMDTP (dimethyldithiophosphate), DEP (diethylphosphate), DETP (diethylthiophosphate), and DEDTP (diethyldithiophosphate)) (25, 30-32)(8).

Outcome measurements

Two different outcome measurements were used in the studies; one measured neurological impairment and the other assessed depressive symptoms. Of the 243 studies, 198 used cognitive function tests to investigate negative neurologic influencess caused by OPs exposure.

Associations between outcome and exposure

Ten of the 198 studies that investiginged cognitive impairment mentioned that at least one measure outcome showed more impairment in the exposed groups;, however, these observations were not significant (P-<0.05). Seveni~~x~~ of the studies reported some significant positive associations of exposure with poor outcome (P-<0.05);, however, even in these cases, the significant decrements were observed only in some of the neurologic tests, mainly in the Digit Span and Santa Ana Dexterity tests. Indeed, there are several versions of these neurologic tests and the significance of the scores often depended on the versions of the tests that were used. ~~Five studies used the Neurobehavioral Evaluation System (NES), f~~Fiveour studies used the Wechsler Adult Intelligence Scale (WAIS or WAIS-R) (34, 35), four studies used the Neurobehavioral Evaluation System (NES) (36)~~(35),~~ two studies used the World Health Organization

Neurobehavioral Core Test Battery (NCTB) (37, 38)~~(37)~~, and the remaining eight ~~four~~ studies used their own scales.

Five studies adopted depressive symptoms as outcome measurements, as shown in ~~the~~ Table2;~~,~~ however, the symptoms used in the studies were not standardized.

Table~~-~~2 ~~The~~ Summary ~~table~~ of depressive symptoms used as outcomemeasurements

Reference	~~Obtained~~ Results obtained	Impact of outcomes
Beseler et al 2006(10)~~(10)~~	Depression due to doctor's diagnosis was not significantly related to low (OR 1.09; 95%CI 0.91, 1.31) or high (OR 1.09; 95%CI 0.91, 1.31) cumulative exposure.	-
Levin et al 1976(23)~~(26)~~	Anxiety score of the pesticide applicators was significantly higher (P<0.05) than that of the farmers. However, there was no significant difference in measures of depression.	++
London et al 1998(24)~~(15)~~	Dizziness, sleepiness, and headache ~~–~~had a significantly higher overall neurological symptom score (P<0.05).	++
Misra et al 1985(26)~~(28)~~	Common symptoms were Headache (59%), giddiness (50%), ocular symptoms (27%), and paresthesia (18%) and no neurologic change was seen.	-
Ohayo-Mitoko et al 2000(27)~~(29)~~	A significant change in symptom prevalence was found for the respiratory (2.48% CI (0.78, 5.38) and central nervous system (2.56% CI (0.99, 6.62), but in terms of skin~~,~~ symptoms~~stematic~~, and eye symptoms, there was no statistically significant change.	++

OR=Odds Ratio ++: Statistically significant (P~~p~~<0.05), -: Not statistically significant

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Statistical analysis

Sixteen studies used logistic regression, and the remaining eight~~seven~~ used other

statistical tests including X^2-test and t-test. Only one study adjusted for sex in the logistic regression. Fourteen~~Thirteen~~ ~~out~~ of the 2~~43~~ studies adjusted for age, and 1~~21~~ adjusted for education in the~~–~~ statistical ana~~l~~lysis~~logistic regression~~. However, only five studies adjusted for alcohol consumption before carrying out the statistical analysis, and ~~, Further,~~ only two studies adjusted for first language.

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Methodological quality appraisal

Based on NOS, ~~F~~five~~our out~~ of the 2~~43~~ studies were of very good quality, 10 were of good quality, and the remaining nine were either satisfactory or unsatisfactory. Most ~~of the bad quality~~ studies with unsatisfactory scores~~quality~~ either were carried out before 1990 or were performed in some of the less developed countries. In particular, the methods of recruitment of subjects, controlling for confounders, and outcome assessment were not appropriate. For example, in some~~ of the~~ studies, all of the participants were volunteers ~~(~~(14, 28)~~24, 30)~~ and in another study, the subjects were not representative of the community from which they were recruited (factory workers) (29)~~(31)~~. In addition, in the unsatisfactory studies, how the outcome was assessed was not described~~ in the unsatisfactory studies~~, and ~~some of the~~ methods needed to avoid confounders such as stratification and regression were not used. ~~On the other hand,~~

~~n~~None of the cohort studies were assessed as very good quality because most of them did not have a long enough follow-up duration (in five studies, the duration was less than six months) and the selected subjects were not fully representative of the target community. Moreover, the methods of outcome assessment were not described in most of the cohort studies.

Data synthesis ~~and meta-analysis~~

~~As shown in Figure 1 and 2, a meta-analysis was carried out using the reported mean scores for the implemented neurobehavioral test; however, because the investigators used different scoring systems, meta-analysis was difficult.~~ The results of the neurologic tests used in the studies are~~were~~ summarized in Table3. As can be seen ~~in the Table3~~, the test batteries differed from ~~each~~ study to study. The commonly used test~~s~~ batteries in NCTB, NES, and WAIS were Symbol-Digit and Digit Span Forward and Backward. However, some studies that adopted NES and WAIS to measure neurologic impairment ~~(Table3)~~ implemented only a few subsets in the trials. Among the 13~~five~~ studies that us~~ed~~ing a Symbol-Digit test, ~~each~~ four~~three~~ used NES and u~~U~~nknown tests, ~~each~~ ~~WAIS,~~ two used WAIS and WAIS-R ~~and unknown tests~~, and one ~~was~~used a Polish NCTB. ~~For~~Among the studies that used Digit Span~~, there were f~~Forward and

bBackward tests, some studies performed both tests, butwhile the others did eitheronly one of the tests as shown in Table3 test, two studies used NES and WAIS in the forward tests and two WAIS in the backward tests. Overall As a result, there were only four of the studies that used the same test battery in NES and WAIS , respectively, and it was impossible to perform a meta-analysis for neurological test batteries. Because there were only two studies in each Digit Span test, a meta-analysis would not be very useful, and so a meta-analysis for the Digit Span tests was not carried out and only a meta-analysis for NES and WAIS Symbol Digit tests was performed. In terms of Symbol Digit (NES), slight positive association can be seen (Figure 1), while Figure 2 showed that there was no difference in mean score of Symbol Digit WAIS between the exposed and control groups. Although the three studies apparently used the same scoring systems, one of the scores was completely different from the scores in the other two studies. For example, the scores in the study byof Stephens et al. (31) were 24.22 and 21.01 in the exposed and the control groups, respectively (30)(17), whereas the scores reported byof Daniell et al. and Stephens et al. were much lower and between 2.23 and 3.55 (12, 20, 31)(16, 17, 20). Similarly, the mean scores reported by Bazylewicz-Walczak et al. (215) were higher, 45.50 and 49.40, while the mean scores reported in the other studies were smaller, 2.28 and 2.23 in the WAIS (25) (24)(27). In

consideration of insufficient number of studies and possible systematic differences in the population characteristics and/or in the measurement procedures between the studies, we decided not to conduct a meta-analysis.

Table 3 ~~The s~~Summary ~~table~~ of the neurologic test batteries used in some of the studies~~battery tests~~

Reference	Types of neurologic tests	Symbol Digit	Digit Span	Santa Ana	Simple Reaction Time	Syntactic Reasoning(s)
Bazylewicz-Walczak et al 1999(21)~~(23)~~	Polish NCTB/WAIS (Symbol Degit)	nd	nd	nd	**~~+~~	nd
Cole et al 1997(11)~~(33)~~	NCTB	nm	nm	nm	nd	nd
Daniell et al 1992(12)~~(20)~~	NES	*~~-~~	nd	nd	nd	nd
Farahat et al 2003(14)~~(24)~~	Unknown	***~~++~~	***~~++~~(f)[1]~~*~~ ***~~++~~(b)[2]**	nd	nd	nd
Fiedler et al 1997(15)~~(34)~~	WAIS-R	*~~-~~	*~~-~~	nd	***~~++~~	nd
London et al 1997(16)~~(18)~~	WAIS-R	nm	nm	***~~++~~	nm	nd
Maizlish et al 1987(25)~~(27)~~	WAIS	~~1/~~***~~++~~	nd	nd	nd	nd
Roldan-Tapia et al 2005(18)~~(14)~~	WAIS	***~~++~~ [3]~~*~~	***~~++~~ [3]~~*~~	nd	nd	nd
Ross et al 2010(17)~~(16)~~	WAIS	nd	***~~++~~	nd	nd	nd
Rothlein et al 2006(32)	Unknown	*	*(f)[1] ***(b)[2]	nd	*	nd
Srivastava et al 2000(29)~~(31)~~	Unknown	***~~++~~	***~~++~~	nd	nd	nd
Steenland et al 2000(30)~~(11)~~	NES	*~~-~~	*~~-~~	nd	*~~-~~	nd
Stephens et al	Unknown	***~~++~~	*~~-~~	nd	***~~++~~	**~~+~~

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1995(19)(12)						
Stephens et al	NES/ACT	nm	nm	nd	nm	nm
1996(31)(17)						
Stephens et al	NES/ACT	*-	*-	nd	*-	***++
2004(1)(16)						(ACTS)

***++: P<0.05, **+: 0.05≦P<0.1, *—: P>0.1

The eExposed groups were slower or had poorer outcomes than the control groups

[1] 1: (f) Digit Span fForward

[2] 2: (b) Digit Span bBackward

[3] †: The article did not mention wWhether the obtained results were positive or negative was not reported in the studyies

nd: The sSubsets of neurological tests were not performed

nm: Although the sSubsets of neurological tests were performed but P-values were not mentioned in the articlereported

*(f) Digit Span forward, **(b) Digit Span backward

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DISCUSSION

The systematic keyword and manual searchesresults showed that there were of the published literature identified 243 epidemiological studies thatwhich examined the relationship between OPs and CNS by systematically searching. When the relevant information was assessedcomparing the selected studies by each item, two main findings were obtained,; one wasis the method of exposure assessment, and the other wasis the method used for the outcome measurement. With respect toFor exposure assessment, the matter of measurement methods wereas categorized asinto three: direct, indirect, and a combination of both methodsdirect and indirect. For the On the other hand, in terms of outcome measurements, there seemed to be two main

assessments~~ways~~ were used,~~to gauge~~ neurologic impairment and depressive symptoms.

Exposure assessment

Exposure assessment was not used for group allocation in all the studies;~~,~~ ~~and~~rather, it was implemented to measure how much subjects were exposed and the outcomes of the neurobehavioral tests. D~~Each study used d~~ifferent exposure assessment methods were used in each study, which made it difficult to accurately compare the studies. In addition, there seemed to be methodological imperfections in both the direct and indirect methods. For example~~To illustrate~~, in one study, an interview~~s~~ and questionnaire~~s~~ were used ~~in the indirect method, though, one study~~for ~~the~~ recruited subjects over 60 years old who had been retired for 11 years ~~since their retirement~~ (17)~~(21)~~. This method is subject~~has the potential of causing~~~~In this study,~~ to recall bias ~~could be a problem~~ because the rate of cognitive impairment is likely to have increased as the subjects aged ~~put on years~~. ~~This could lead to inaccuracy of exposure assessment~~. However, other indirect methods, especially extensive history records of pesticide use could be considered as a proxy of how much OPs might have accumulated in the body, thus records of this type can be used to estimate the amount of OPs by long-term exposure, even though there may be some recall bias. ~~With respect to~~For the direct method~~s~~, ~~there were several ways to~~

detect OPs. Although some studies used DPAbiomarkers useor dsuch as urinary metabolites as an indicator of exposure was used, as an exposure index in the study; however, DPA is metabolized rapidly and excreted from bodies (6)(7). Therefore, measuring urinary analysis was not a perfect way to assess OPs exposure, oOn the contrary, it seemed that measuring AChE levels was the most reliable way to assess the amount of OP exposure, because the blood AChEcholinesterase levels needstake approximately one week to becomes normal by being synthesized into a new molecular of AChE, which takes around a week (39)(35). hHence, althoughthe amount of OP exposure within one week can be accurately measured by AChE inhibition level in blood, but the blood AChE levelsthis cannot be be used to assessed the amountaccumulation of of OPs exposure accumulated in body tissues over for a long time, it Thus, direct method using the levels of AChE in blood is appropriate forcan be used to assessing short-term exposure, however, it is not for long-term exposure. On the contrary, iIndirect methods, especially extensive history records of pesticides such as structured interview and questionnaire could be a proxy helpful to grasp the past information about OPs usehow much OPs were accumulated in the body, even though there may be some recall bias. In order tTo minimize measurement errors, a mixed method for the assessment of short-term and long-term exposure should be established.

~~a it is desired that standardized measurement method should be established for further research. a combination of direct and indirect methods should be used.~~

Outcome assessment

The main problem in analyzing the outcome measurements was the inconsistencies in ~~the results of~~ neurologic test batteries~~ were not consistent~~~~differed from each study~~. V~~,~~ ~~and even if the same test battery was used, the types of tests such as NES and WAIS were different.~~ ~~To elaborate, as shown in Table 3, three studies adopted WAIS and four used NES as outcome assessment, and since there were v~~various versions of the neurologic tests were used in the studies and ~~battery tests including WAIS and WAIS-R,~~ the content of the tests ~~slightly~~ differ slightly ~~from~~in each study (Table3). Therefore, only a few tests were common across some of the studies, which made it difficult to compare the studies. Further, a meta-analysis could not be applied because of the insufficient ~~a small number of~~ number of studies. M~~Performing a m~~eta-analysis could have been performed ~~might be possible~~ by dividing the results into subgroups~~,~~; however, the ~~A meta-analysis using~~ results ~~that would be obtained from the meta-analysis~~ could

be highly misleading due tobecause of loss of power of studies (40) and cause sampling and publication biases. a small number of studies has the potential of causing sampling and publication bias due to small effect size, and even if a meta-analysis was implemented, the reliability would be low. Similarly, iIn terms of depressive symptoms, the outcomes assessment was againere different infrom each study. , fFor instance, one study usedhad the proportion of headaches, while the another used that of dizziness and sleepiness as the main outcomes. To gain better insights into whether precise conclusion that occupationalcumulative OP exposure can negatively affect the human CNS or not, at the very least, avoid these problems, aneurologic test batteriesbattery tests, at least, should be standardized outcome measurement and integrateda guidelines for measuring of neurologic symptomsimpairment should be set for all future epidemiological studies. As with exposure assessment, a similar problem can be seen in outcome assessment, for example, five out of the 23 studies adopted depressive symptoms as outcome measurements (Table 2). On the other hand, the remaining 18 studies used neurologic battery tests such as NES and WAIS. Thus the main problem in the outcome measurements is that comparison between the studies could not be done easily, because neurologic battery tests differed by each study. To elaborate, as shown in Table 3, three studies adopted WAIS and four used NES as outcome assessment, and since there were

~~various versions of neurologic battery tests including WAIS and WAIS-R, the content of the tests slightly differ from each study.~~ Furthermore, although some studies mentioned ~~about~~ the possible relationship between OPs exposure and confounding factors such as age and education, ~~they did not perform~~ statistical tests between the exposed and control groups were not performed in these studies. These inconsistencies ~~things obviously~~ make it difficult to compare the ~~outcomes of~~ neurologic impairment outcomes among the studies. ~~In addition, even in the same neurologic battery test, there are a variety of subtests such as Symbol Digit and Digit Span to measure neurologic impairment. The studies selected some subtests in their trials, hence there were few studies left to precisely compare. As a consequence, although the meta-analysis was carried out using the results of Symbol Digit, it was not enough to determine whether or not there was a statistically significant relationship. Similarly, in terms of depressive symptoms, outcomes were different from each study, for instance, one study had the proportion of headache, while the other used that of dizziness and sleepiness as main outcomes. Thus, neurologic battery tests, at least, should be standardized for further epidemiological research. If not, it could be difficult to gain precise conclusion that cumulative OP exposure can negatively affect human CNS or not.~~

Study design

Although 176 ~~Sixteen~~ of 243 ~~the~~ studies were cross-sectional studies, ~~and six were cohort studies.~~ ~~L~~longitudinal or cohort studies are more appropriate, ~~desirable rather than cross-sectional studies for three main reasons: one, in cross-sectional studies, it is difficult to confirm whether or not the disease preceded the exposure; two,~~ ~~B~~because ~~the~~ ~~outcome conditions in cross-sectional studies are too short lasting (36); and three, cross-sectional studies are suitable for investigating at a certain point, but they are not appropriate for mid-term studies. Especially,~~ agricultural work using pesticides is easily influenced by seasonality, ~~and o~~One research regarding reproductive health by OPs exposure stated that sperm concentration and counts are negatively affected ~~on~~in spring, peak season, ~~spring,~~ rather than winter (5)~~(6)~~. Therefore, ~~T~~the ~~result~~effect ~~on~~of the the CNS ~~neurobehavioral tests may~~could also be affected by seasonality; ~~therefore, cohort studies are ideal to assess the influence of occupational OPs exposure than cross-sectional.~~

Sources of ~~P~~possible biases

Only published studies written in English were searched, thus publication bias could have occurred. In future studies, non-English studies and unpublished studies should be

included to reduce publication bias. ~~If foreign workers are included i~~In ~~the~~ trials that included foreign workers, ~~their~~ first language and education levels ~~should~~could be considered as possible biases. ~~B~~ because there is a possibility that ~~the~~ non-native subjects ~~cannot~~did not fully understand the content and instructions ~~of~~for the tests, which could lead to them obtaining a lower score than ~~that of~~ native speakers. Additionally, the education systems in developed and less developed countries could be very different. Nowadays, developed countries such as USA and the ~~g~~Gulf countries have accepted foreign workers ~~from India and South American countries~~ as an important part of the work-force (12, 32, 41)~~(20, 37, 38)~~. These factors needed to be adjusted carefully in the sampling and analytical stages of the study; ~~H~~however, ~~in this systematic review, there were~~ only two of the selected studies ~~to~~ mentioned ~~about~~ first language in their statistical analyses ~~inclusion and exclusion criteria~~ (12, 31)~~(17, 20)~~. ~~OSince first language could influence the outcomes, it should be one of the factors to be considered when selecting subjects. Furthermore, when migrants and foreign labourers are included in the studies, education system is a point that we have to pay attention. Because education system between developed and less developed countries could be largely different. Hence, it is necessary to be careful when the results between subjects who come from different countries are compared. Additionally, o~~occupations could ~~be a~~

~~factor of~~also contribute to selection bias~~,~~ because, for example, a police officer or a~~and~~ construction worker~~s~~ would have a higher ~~possibility~~probability of experiencing ~~the~~ loss of consciousness due to accidents ~~of their jobs~~than workers with different occupations (17)~~ (21)~~.

Possible confounders

~~Age and social cultural factors are known as common confounding factors, though, not all studies adjusted them in the analysis. These factors could easily influence the results; hence they should be adjusted for further trials. Moreover,~~ Apart from common confounders such as age and education, ~~since~~ head injury and alcohol consumption could be other confounders, because~~have a probability of negatively affecting neurologic battery tests,~~ they can cause neurologic impairment due to memory deterioration.~~they should be treated as potential confounders as well.~~ Although some of the studies adjusted for alcohol consumption in the analysis (10, 12, 16, 24, 25)~~(10, 15, 18, 20, 27)~~, no study adjusted for head injury.~~However, the results showed that there was no study to adjust head injury in the logistic regression, on the other hand, there were some studies to adjust alcohol consumption in the analysis (10, 15, 18, 20, 27). Apart from these factors~~Furthermore, ~~participants'~~ nutrition status including vitamin

deficiency can~~is~~ also be relevant to the outcome of neuropsychological tests (16, 24)~~(15, 18)~~. Thus, ~~all~~ factors other than the common confounders that could negatively~~an~~ affect ~~measurements of~~ cognitive function should be adjusted for in the analysis.

~~Limitations~~Strengths and limitations of this review~~study~~~~weaknesses~~

~~Strengths~~

A major strength of this systematic review is that the characteristics of the selected studies were summarized using tables, and limitations of the exposure and outcome assessments used in these studies were ~~mainly~~ identified mainly on the basis of the constructed tables. Furthermore, the systematic review allowed us to propose recommendations that will be useful for standardizing future epidemiological research.

~~Weaknesses~~

All of the selected studies were relevant to occupational OPs exposure; however, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides that are commonly used in agriculture are usually mixtures of different pesticides, which are used to increase their effect. Four of the 2~~3~~ selected studies used a combination of OPs, organochlorines, carbamates, and fungicide; hence, the effect of only occupational OPs exposure could not be measured in these studies.

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InAlthough all of the studies which were collected in this systematic review were relevant to occupational OP exposure, some of them included other pesticides such as carbamates, fungicides, and herbicides. Pesticides usually are mixed with another type of pesticides to make their effects stronger, and this is the common in agriculture. In theis systematic review, four out of 23 studies were not single OPs exposure and they used a combination of OPs, OCs carbamates and fungicide,. which complicated Therefore, it may be quite difficult to measure the effect of only occupational OP exposure.

Of these studies, the outcome assessments18 assessed neurological or neuropsychological impairment using IQ tests. However, since the authors used the different neurological types of tests were usedbattery tests such as NCTB, NES, and WAIS, consequently, the lack of pooling evidence meant that there were only a few common tests including Digit Span and Symbol digit tests across the studies, comparisons among the studies became extremely difficult , furthermore, which made the comparison of the included studies more difficult. Hence, a meta-analysis wascould not be performed applied, to the two tests, but it is Small effect size due to a small number of studies may cause sampling and publication bias. and even if a meta-analysis is applied, it would be unreliable obvious that studies which can be appraised are

limited. In order to completely assess neurological impairment, there is necessity of the standardized tests battery for measuring neurological impairmentit is desirable that the same neurobehavioral test battery be used in a large number of studies. FurthermoreIn addition, the exclusionexcluding of studies written in languages other than English is another limitation of this review, and literature retrieval by only the first author could have introduced some bias into the selection of the studies.one of anotherreview

CONCLUSION

AlthoughWhile some studies indicated negative influence on the human CNS based on the results of neurobehavioral or neuropsychological test batteries, the others did not. Hence, enough consistent results were not obtained to determine whether or not occupational OPs exposure could be harmful on the human CNS. the suggestive evidence for neurobehavioral test battery is inconsistent, there was a slight positive relationship of poor outcome implying that occupational exposure to OPs could be harmful for the CNS of the human. The evidence was weak in particular because some studies showed that there was a negative relationship of OPs with poor outcome. In

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addition, since tThe test items tested inof the neurologicalbehavioral or neuropsychological test batteries,y and the estimates of OPs exposure were inconsistent because they depended on the preferences of the investigators, thus they were inconsistent.only a few items were common across the studies. Consequently, because there were only a few studies left, a meta-analysis could not be performed. for the meta-analysis; indeed, there were a few items which could be compared. For future studies, the neurobehavioral andor neuropsychological test types, test batteries,method used to ey should be standardizsed in order to ensure adequate quality and to make it more possible to pooling the evidence from a large number of the studies for future analysis.

For future studies, it would be best to standardize the neurological and neuropsychological test types, test batteries, and the methods used to measure OPs, to enable precise comparisons of results and pooling of evidence from a large number of studies for future analyses. However, this may be difficult to achieve in practice because OPs are used in differing settings around the world, and education systems vary considerably between countries.

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FOOTNOTES

Contributors NT conceived the study design and participated in protocol development, literature searching, data extraction, data analysis, and drafted the manuscript. MH critically reviewed the draft and contributed to the manuscript revisions.

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Ethical approval Systematic review.

Provenance and peer review Not commissioned, externally peer reviewed.

Data sharing statement No additional data are available.

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FIGURE LEGEND

Figure 1: Flow diagram of search and review process

~~Figure1 represents how the selected articles were searched. After electric search was conducted with restriction of published year, human, and English, a manual search of titles and abstracts was carried out. As a result, the remaining 21 studies were fully reviewed, and 12 studies met the inclusion and exclusion criteria. Another 12 studies were found by hand search.~~

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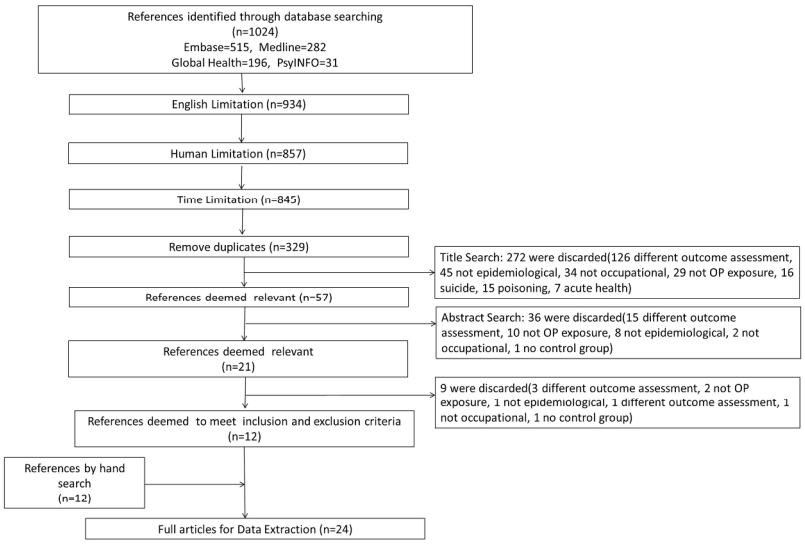

The figure1 represents the flow of database search and review process.
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Appendix A

The Appraisal Standard of Newcastle/Ottawa Scale

Selection

1) Representativeness of the exposed group/cohort

a) Truly representative of the average farmers or pesticides applicators in the community *

b) Somewhat representative of the average farmers or pesticides applicators in the community*

c) Selected group of users (e.g. factory workers, volunteers)

d) No description of the derivation of the group

2) Selection of the non-exposed group/cohort

a) Drawn from the same community as the exposed group*

b) Drawn from a different source

c) No description of the derivation of the non-exposed group

3) Ascertainment of exposure

a) Secure record (e.g. biomarkers)*

b) Structured interview or questionnaire*

c) Written self reports

d) No description

4) Demonstration that outcome of interest was not present at start of study *(Cohort Studies Only)*

a) Yes*

b) No

Confounder

1) Comparability of groups on the basis of the design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor* (e.g. alcohol consumption, smoking, and first language)

Outcome

1) Assessment of outcome

a) Independent blind assessment*

b) Record linkage*

c) Self reports

d) No description

2) Was follow-up long enough for outcomes to occur *(Cohort Studies Only)*

a) Yes (select an adequate follow up period for outcome of interest)*

b) No

3) Adequacy of follow up of cohorts *(Cohort Studies Only)*

a) Complete fellow up – all subjects accounted for*

b) Subjects lost to follow up unlikely to introduce bias – small number lost - > 70% follow up, or description provided of those lost*

c) Follow up < 70% and no description of those lost

d) No statement

Case Control Studies:

Selection

1) Is the case definition adequate?

a) Yes, with independent validation*

b) Yes, e.g. record linkage on self reports

c) No description

2) Representativeness of the cases

a) Consecutive or obviously representative series of cases*

b) Potential for selection biases or non stated

3) Selection of Controls

a) Community controls*

b) Hospital controls

c) No description

4) Definition of Controls

a) No history of disease (endpoint)*

b) No description of source

Confounder

1) Comparability of cases and controls on the basis of design or analysis

a) Study controls for age and education*

b) Study controls for any additional factor*

Exposure

1) Ascertainment of exposure
a) Secure record (e.g. biomarkers)*
b) Structured interview where blind to case/control status*
c) Interview not blinded to case/ control status
d) Written self reports or medical record only
e) No description

2) Same method of ascertainment for cases and controls
a) Yes*
b) No

3) Non-Response rate
a) Same rate for both groups*
b) Non respondents described
c) Rate different and no designation
*: plus one point

There are five items in cross-sectional studies and eight items in cohort and case control studies, respectively. The quality of the studies was defined as follows.

Cross-sectional Studies:
Very Good Studies: 5 points
Good Studies: 4 points
Satisfactory Studies: 3 points
Unsatisfactory Studies: 0 to 2 points

Cohort / Case control Studies:
Very Good Studies: 7 to 8 points
Good Studies: 5 to 6 points
Satisfactory: 4 points
Unsatisfactory Studies: 0 to 3 points

Appendix B

Table1 Quality Appraisal (Cross-sectional Studies)

Selection	Cole et al 1997	Dassanayake et al 2009	Farahat et al 2003	Fiedler et al 1997	Korsak et al 1977	Levin et al 1976
1) Representativeness of the exposed group						
a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	a) (+1)	b) (+1)	c) (0)	a) (+1)	b) (+1)	b) (+1)
2) Selection of the non exposed group						
a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	b) (0)	b) (0)	a) (+1)	a) (+1)	b) (0)
3) Ascertainment of exposure						
a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	d) (0)	a) (+1)	b) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis	b) (+1)	- (0)	a) (+1)	- (0)	- (0)	- (0)
a) Study controls for age and						

education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)						
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	a) (+1)	b) (+1)	d) (0)	b) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	2/5 Unsatisfactory	2/5 Unsatisfactory	4/5 Good	3/5 Satisfactory	3/5 Satisfactory

Continued…

Table1 Continued

Selection	London et al 1997	London et al 1998	Maizish et al 1987	Rodnitzky et l 1975	Roldan-Tapia et al 2005
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	b) (+1)	a) (+1)	c) (0)	c) (0)	a) (+1)
2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	c) (0)	a) (+1)

3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	b) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	b) (+1)	b) (+1)	b) (+1)	- (0)	a) (+1)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	c) (0)	a) (+1)	d) (0)	a) (+1)
Overall Score	5/5 Very Good	4/5 Good	4/5 Good	1/5 Unsatisfactory	5/5 Very Good

Continued…

Table1 Continued

Selection	Rothlein et al 2006	Srivastava et al 2000	Steenland et al 2000	Stephens et al 1995	Stephens et al 1996	Stephens et al 2004
1) Representativeness of the exposed group a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the group	b) (+1)	c) (0)	a) (+1)	a) (+1)	a) (+1)	a) (+1)

2) Selection of the non exposed group a)Drawn from the same community as the exposed group b)Drawn from a different source c) No description of the derivation of the non exposed group	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	b) (+1)	a) (+1)	a) (+1)	c) (0)	a) (+1)	b) (+1)
Confounder 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption, smoking, and first language)	a) (+1)	- (0)	b) (+1)	b) (+1)	b) (+1)	- (0)
Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	d) (0)	a) (+1)	b) (+1)	d) (0)	b) (+1)
Overall Score	5/5 Very good	2/5 Unsatisfactory	5/5 Very Good	4/5 Good	4/5 Good	4/5 Good

Table2 Quality Appraisal (Cohort Studies)

Selection	Albers et al 2004	Bazylewicz-Walczak et al 1999	Daniell et al 1992	Ohayo-Mitoko et al 2000	Misra et al 1985	Ross et al 2010
1) Representativeness of the exposed cohort a) Truly representative of the average farmers or pesticides applicators in the community b)Somewhat representative of the average or pesticides applicators in the community c) Selected group of users d) No description of the derivation of the cohort	c) (0)	c) (0)	a) (+1)	b) (+1)	c) (0)	a) (+1)
2) Selection of the non exposed cohort a)Drawn from the same community as the exposed cohort b)Drawn from a different source c) No description of the derivation of the non exposed cohort	b) (0)	a) (+1)	b) (0)	a) (+1)	b) (0)	b) (0)
3) Ascertainment of exposure a) Secured record (e.g. biomarkers) b) Structured interview or questionnaire C) Written self report d) No description	a) (+1)	a) (+1)	a) (+1)	b) (+1)	a) (+1)	b) (+1)
4)Demonstration that outcome of interest was not present at start of study a) Yes b) No	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)	a) (+1)
Confounders 1) Comparability of groups on the basis of the design or analysis a) Study controls for age and education b) Study controls for any additional factor (e.g. alcohol consumption,	- (0)	a) (+1)	b) (+1)	- (0)	a) (+1)	a) (+1)

smoking, and first language)						

Continued…

Table2 Continued

Outcome 1) Assessment of outcome a) Independent blind assessment b) Record linkage c) Self report d) No description	b) (+1)	d) (0)	d) (0)	c) (0)	d) (0)	d) (0)
2) Was follow-up long enough for outcomes to occur a) Yes (select adequate follow up period for outcome of interest b) No	b) (0)	b) (0)	b) (0)	b) (0)	b) (0)	a) (+1)
3) Adequacy of follow up of cohorts a) Complete follow up-all subjects accounted for b) Subjects lost to follow up unlikely to introduce bias- small number lost- >70% follow up, or description provided of those lost c) Follow up rate<70% and no description of those lost d) No statement	b) (+1)	a) (+1)	a) (+1)	c) (0)	d) (0)	d) (0)
Overall Score	4/8 Satisfactory	5/8 Good	5/8 Good	4/8 Satisfactory	3/8 Unsatisfactory	5/8 Good

Table3 Quality Appraisal (Case-control Studies)

Selection	Beseler et al 2006
1) Is the case definition adequate?	
a) Yes, with independent validation b) Yes, e.g. record linkage or based on self reports C) No description	b) (0)
2) Representativeness of the cases	
a) Consecutive or obviously representative series of cases b) Potential for selection biases or not stated	a) (+1)
3) Selection of Controls	
a) Community controls b) Hospital controls C) No description	a) (+1)
4) Definition of Controls	
a) No history of disease (endpoint) b) No description of source	a) (+1)
Confounders 1) Comparability of cases and controls on the basis of design or analysis	
a) Study control for age and education b) Study controls for any additional factor	b) (+1)
Exposure 1) Ascertainment of exposure	
a) Secure record(biomarkers) b)Structured interview where blind to case/control status c) Interview not blinded to case/control status d) Written self report or medical record only	d) (0)

e) No description	

Continued…

Table3 Continued

2) Same method of ascertainment for cases and controls a) Yes b) No	a) Yes
3) Non-response rate a) Same rate for both groups b) Non respondents described c) Rate different and no designation	b) (0)
Overall Score	5/8 Good

MOOSE Checklist		
Section/Topic	**Checklist items**	**Check**
Title	• Identify the study as a meta-analysis (or systematic review)	X
Abstract	• Use the journal's structured format	X
Introduction (Present)	• The clinical problem	X
	• The hypothesis	X
	• A statement of objectives that includes the study population, the condition of interest, the exposure or intervention, and the outcome(s) considered	X
Sources (Describe)	• Qualifications of searchers (eg, librarians and investigators)	X
	• Search strategy, including time period included in the synthesis and keywords	X
	• Effort to include all available studies, including contact with authors	X
	• Databases and registries searched	X
	• Search software used, name and version, including special features used (eg, explosion)	X
	• Use of hand searching (eg, reference lists of obtained articles)	X
	• List of citations located and those excluded, including justification	X
	• Method of addressing articles published in languages other than English	X
	• Method of handling abstracts and unpublished studies	X
	• Description of any contact with authors	X
Study Selection (Describe)	• Types of study designs considered	X
	• Relevance or appropriateness of studies gathered for assessing the hypothesis to be tested	X
	• Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	X
	• Documentation of how data were classified and coded (eg, multiple raters, blinding, andinterrater reliability)	X
	• Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	X
	• Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	X
	• Assessment of heterogeneity	N/A
	• Statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models,or cumulative meta-analysis) in sufficient detail to be replicated	N/A
Results (Present)	• A graph summarizing individual study estimates and the overall estimate	X
	• A table giving descriptive information for each included study	X
	• Results of sensitivity testing (eg, subgroup analysis)	X
	• Indication of statistical uncertainty of findings	X
Discussion (Discuss)	• Strengths and weaknesses	X
	• Potential biases in the review process (eg, publication bias)	X
	• Justification for exclusion (eg, exclusion of non–English-language citations)	X
	• Assessment of quality of included studies	X
	• Consideration of alternative explanations for observed results	X
	• Generalization of the conclusions (ie, appropriate for the data presented and within the domainof the literature review)	X
	• Guidelines for future research	X
	• Disclosure of funding source	X
N/A: Not Applicable		